BLOOD FLOW RESTRICTION: REHABILITATION TO PERFORMANCE

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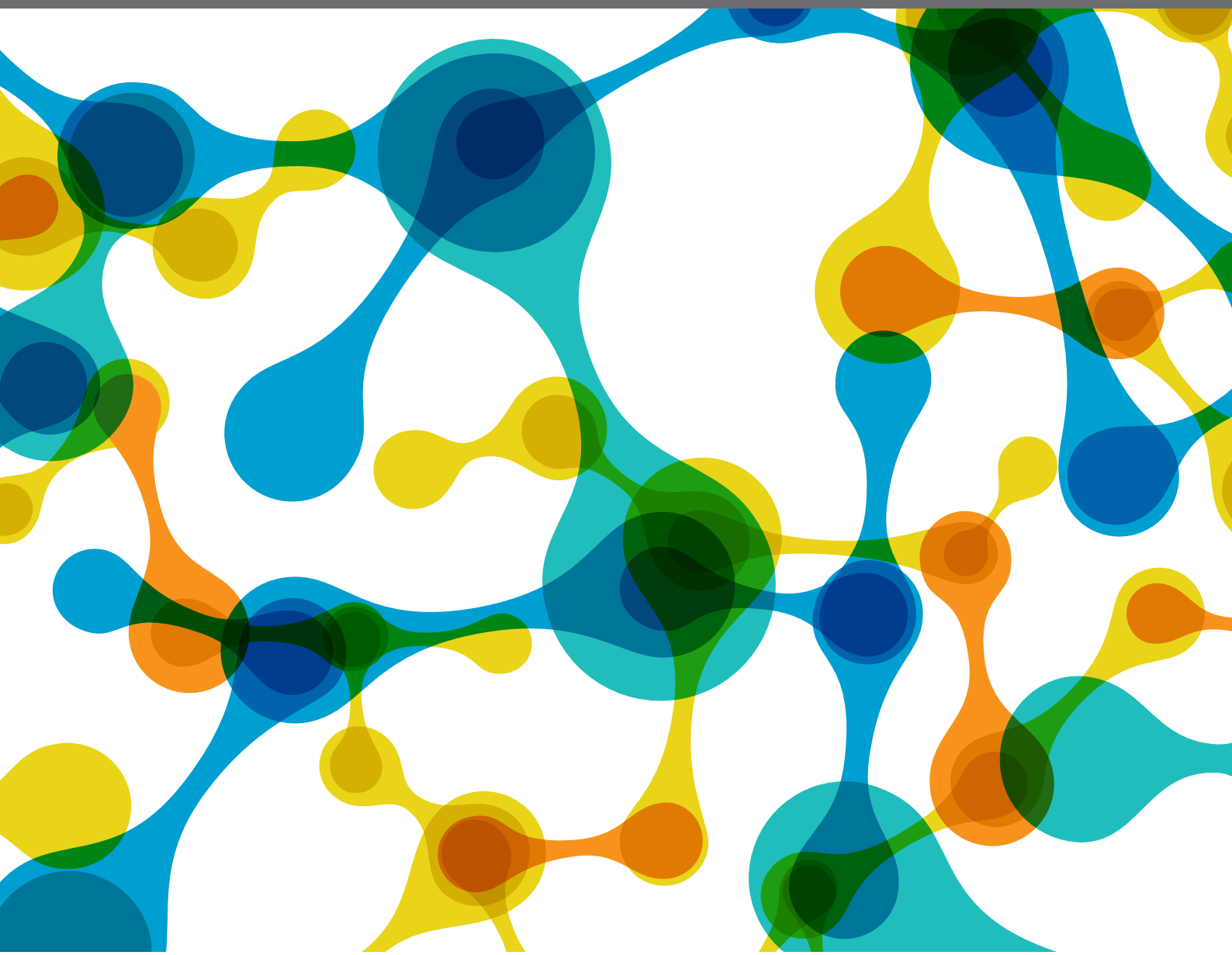

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BLOOD FLOW RESTRICTION: REHABILITATION TO PERFORMANCE

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EDITORIAL
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Editorial: Blood Flow Restriction: Rehabilitation to Performance

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Editorial on the Research Topic

Blood Flow Restriction: Rehabilitation to Performance

INTRODUCTION

The manipulation of limb blood flow via the use of specialized cuffs, bands, or tourniquets may be used to target specific acute physiological responses or chronic training induced adaptations affecting strength, hypertrophy, or aerobic exercise efficiency and performance. This manipulation may be broadly captured by the techniques of (a) blood flow restricted exercise, and (b) ischemic pre-conditioning, and as such these form the basis/focus of this Research Topic.

Blood flow restriction (BFR) exercise is a relatively novel training technique used for increasing skeletal muscle mass and strength. This BFR technique restricts muscle blood flow through the application of an external pressure, typically using a pneumatic tourniquet/cuff system applied to the most proximal section of the upper or lower limbs. Inflation of the cuff produces a mechanical compression of the underlying tissues leading to a full or partial restriction of both venous and arterial vasculature. This variable degree of blood flow restriction likely affects a reduction in venous return while creating tissue hypoxia distal to the cuff, with the magnitude of both these effects being modulated by the phase and intensity of muscle contractile activity or exercise. Importantly, the gains in skeletal muscle size and strength with BFR training have been typically demonstrated when using light exercise loads/intensities (e.g., 20–30% one repetition maximum; walking exercise), generating supporting evidence for BFR during voluntary resistance and aerobic exercise, and also passively without exercise. More recent research has also examined the combination of BFR with non-traditional exercise modalities such as whole-body vibration techniques and neuromuscular electrical stimulation. Despite a growing body of evidence in support of beneficial skeletal muscle outcomes as well as functional performance benefits in a range of populations, at present, there is no universal standard method for the application of BFR during exercise (Patterson et al.). Differences exist for cuff type and size, pressures used the method for determination of cuff pressure and the duration of BFR application. Its present uses include rehabilitation from injury and to improve aspects of athletic performance.

Ischemic preconditioning (IPC) is, by contrast, when a tourniquet/cuff is placed on either the arm(s) or leg(s) of participants prior to exercise performance, irrespective of the exercise modality. The application of an IPC protocol pre-activity, which typically includes cyclic occlusion of remote or local skeletal muscle tissue of the limb, may render skeletal muscle of athletes more resistant to fatigue due to similar muscular modifications demonstrated in a clinical setting. As such IPC is currently being assessed as to its validity as an ergogenic aid in both aerobic and anaerobic exercise performance (Incognito et al., 2016). Furthermore, evidence exists around the use of IPC to assist with recovery from exercise/fatigue and adaptation to training.

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TOPIC CONTENT

This Research Topic accepted 21 articles for publication (18 original research papers, 1 systematic review, 1 review and 1 opinion) written by a total 127 contributing authors. Overall, this topic pools research focused on acute responses and adaptations to blood flow restriction training and ischemic preconditioning. Based on the numerous contributions to this Research Topic we have learned the following:

ACUTE RESPONSES OF BLOOD FLOW RESTRICTION TRAINING

When examining the acute muscle, metabolic and cardiopulmonary responses to blood flow restriction resistance exercise (BFR-RE), Ilett et al. demonstrated a progressive response with increasing applied restriction pressure during BFR-RE that aligned with reduced MVC torque, increased blood lactate and EMG, and reduced muscle oxygenation. From this, the authors concluded that a minimum "threshold" around 60% limb occlusion pressure (LOP) may be necessary for BFR-RE to affect changes in these oft-cited training variables that may be intrinsic for longer term BFR-RE training adaptation. Reis et al. further examined the effect of different relative applied restriction pressures on muscle oxygenation during BFR-RE. Even with light-load BFR-RE (20% 1-RM), all pressures tested (40, 60, 80% LOP) induced muscle microvascular deoxygenation, but only pressures above 60% LOP demonstrated severely restricted reoxygenation during the intervals between exercise sets. Taken together, these two studies suggest higher pressures (60–80% LOP) are needed to provide sufficient acute physiological stimulation that may translate into chronic adaptations when utilized during training.

Scott et al. compared the hemodynamic responses to low-load resistance exercise (LL-RE), low-load BFR-RE and unrestricted high-load (HL-RE) in older women. While cardiac measures were similar between HL-RE and BFR-RE (e.g., cardiac output, stroke volume), the authors demonstrated significantly higher blood pressures (systolic, diastolic, and mean arterial pressure) for BFR-RE compared with both HL-RE and LL-RE, and this vascular stress was greater when more muscle mass was used during the exercise (e.g., leg press vs. leg extension). This reinforces suggestions that caution be exerted when prescribing BFR-RE to certain at-risk populations. Aligned with these cardiovascular effects (Montgomery et al.) examined circulating endothelial progenitor cells (EPCs), which are a vasculogenic subset of progenitors that play a key role in maintaining endothelial integrity. They hypothesized that EPC mobilization may be augmented by the local hypoxia observed with BFR-RE. However, the authors found that BFR-RE impairs this expected rise in circulating EPCs in the post-exercise recovery period compared with non-BFR exercise. Taken together these two acute studies suggest some caution and certainly further research needs to explore the long-term hemodynamic and vasculature effects of BFR-RE.

ADAPTATIONS TO TRAINING WITH BLOOD FLOW RESTRICTION TRAINING

When examining the suitability of BFR for older populations, Cook and Cleary compared the effect of BFR-RE against HL-RE in older adults who were at risk of mobility limitations. Across a 12-week intervention with similar training volumes between the HL-RE and BFR-RE the growth in knee extensor (KE) and knee flexor (KF) CSA was similar. However, KE strength gains were greater in the HL-RE group, most likely due to the greater training load, while training repetitions were greater in the BFR-RE group. Interestingly, when this training load difference was eliminated in the KF, and in fact was "relatively" greater in BFR-RE, resulting in similar gains in KF strength across the intervention. Ultimately, the authors conclude that even in older adults, incorporating systematic load progression throughout training periods should be employed to maximize strength gains. This work is in agreement with previous evidence that suggests BFR-RE shows less gain in muscle strength compared to HL-RE (Loenneke et al., 2012; Hughes et al., 2017; Lixandrão et al., 2018), however the most up to data meta-analysis suggests there to be no difference for both strength and muscle mass (Grønfeldt et al., 2020). Therefore, as per the guidelines in Patterson et al. it is recommended to use BFR-RE under specific circumstances (e.g., post-operative rehabilitation, cardiac rehabilitation, inflammatory diseases, and frail elderly) with a progression back to HL-RE as the ultimate aim.

A number of other studies also examined the differences between high and low load training prescriptions compared to more traditional RE controls. To investigate BFR-RE in a "real world" situation by using whole body resistance exercise, rather than the short-term upper or lower body BFR-RE commonly used in previous research, Brandner et al. employed a 12-wk study with both a training and detraining component. Comparing BFR-RE, HL-RE, LL-RE, or a non-exercise control (CON) the whole body BFR-RE improved both lower- and upper-body strength. Interestingly, given the use of multiple upper- and lower-body exercises across each training session, collectively lasting ∼45 min, these changes were similar to LL-RE, but both groups were still lower in comparison with HL-RE, with all three training groups being greater than CON. This raises a question as to whether long-duration sessions and multiple exercises within a training session dampen the effectiveness of BFR-RE. Following the additional detraining period, whole body strength remained significantly elevated for both BFR-RE and HL-RE, but only the HL-RE group remained higher than all other groups.

Using an innovative training design to examine the mechanisms driving strength gains and hypertrophy with RE, Biazon et al. compared the effects of high mechanical tension training protocols with and without BFR (HL-RE and HL-BFR-RE), and metabolically stressful training protocols induced with BFR [BFR-RE (light-load) and HL-BFR-RE (high-load)] on deoxyhemoglobin concentration, muscle cross-sectional area (CSA), activation, strength, architecture and oedema before and after 10 weeks of training, with BFR released between sets. The authors concluded that mechanical tension and metabolic stress

seem to share the variance of the muscle hypertrophy response under high mechanical tension protocols, while metabolic stress seems to be the main mechanism responsible for muscle hypertrophy when mechanical tension is low.

Aligned with this, Sieljacks et al. found that despite significantly greater strength gains with HL-RE (13–23%) compared with BFR-RE (6–10%), 6-weeks training produced greater increases in myofibrillar muscle protein synthesis, muscle RNA synthesis and total RNA content in both BFR-RE and HL-RE compared with a CON, with no significant differences between the two exercise groups. This study demonstrated that BFR-RE increased long-term muscle protein turnover and ribosomal biogenesis to a similar degree to HL-RE. Extending on this, Groennebaek et al. found that resistance exercise can stimulate mitochondrial biogenesis and respiratory function to support healthy skeletal muscle and whole-body metabolism. Intriguingly, BFR-RE produced similar mitochondrial adaptations at a markedly lower load, than HL-RE. Collectively, these studies support the clinical value of BFR-RE for populations in whom exercise with high loading is untenable.

Jessee et al. studied the effect of BFR-RE on the hypertrophic response to different load and pressure combinations of BFR-RE. They investigated muscular adaptations following resistance training with a very LL-RE alone (15% 1RM/0% LOP), with moderate BFR (15% 1RM/40% LOP), or with high BFR (15% 1RM/80% LOP), and compared them to HL-RE (70% 1RM/0% LOP). With the exception of 1RM, changes in strength and muscle size were similar, regardless of load or restriction. The training volume required to elicit these changes lowered with increased BFR pressure.

Due to the low load nature of BFR-RE there is a potential role of this mode of exercise in the pre-habilitation and rehabilitation of different injuries. Indeed, Žargi et al. demonstrated that short-term preconditioning with BFR-RE attenuated the deterioration of quadriceps muscle endurance in the postoperative period following ACL reconstruction. This enhanced quadriceps endurance was triggered by a combination of augmented muscle fiber recruitment and enhanced muscle perfusion. The latter alludes to a preserving effect of preconditioning with BFR-RE exercise on density and function of quadriceps muscle microcirculation within the first 4 weeks after ACL reconstruction. Alternatively, during the rehabilitation period Ladlow et al. evaluated the efficacy and feasibility of BF-RE training vs. HL-RE on the clinical outcomes of patient's undergoing inpatient multidisciplinary team rehabilitation for lower-limb injury. They found comparable improvements in muscle strength and hypertrophy between BFR-RE and HL-RE following in-patient rehabilitation. The BFR-RE group also achieved significant improvements in functional capacity, suggesting BFR-RE as a rehabilitation tool with the potential to induce positive adaptations without high mechanical loads. As such, BFR-RE could be considered a treatment option for patients suffering significant functional deficits for whom conventional loaded RT is contraindicated.

SAFETY AND EFFICACY

Crisafulli et al. investigated the effect of safety with longer term (1 month) training with BFR-RE. While blood pressure and systemic vascular resistance remained unaltered at rest, the authors found that BF-RE reduced blood pressure during handgrip exercise, thereby suggesting a potential hypotensive effect of this modality of training. However, this reduction in MAP during handgrip exercise seemed not to be mediated by the metaboreflex, which remained unaffected following the training period. Clarkson et al. completed a systematic review to determine whether exercise interventions utilizing BFR were able to improve objective measures of physical function indicative of activities of daily living. Using data from 13 studies they report BFR exercise, including multiple modalities, improved objective measures of physical function indicative of activities of daily living.

The work by Patterson et al. brought together much of the current research from this Research Topic and the literature in general. The authors set out a series of guidelines for BFR exercise, focusing on the methodology, application, and safety of this mode of training to inform practitioners how BFR exercise should be applied. Included with this the authors not only set out guidelines for BFR-RE, but also BFR with aerobic exercise and passive BFR, while providing clear guidance for the pressures, sets, reps etc. that should be used when employing this technique. This work should be regularly updated to keep abreast of any changes and updates in the literature.

ISCHEMIC PRECONDITIONING

The ability of ischemic preconditioning (IPC) to enhance exercise capacity may be mediated through altering exercise-induced blood flow and/or vascular function. To this end, Cocking et al. demonstrated that a single local-IPC (but not remote-IPC) performed 20 min prior to a 30 min handgrip exercise (25% MVC) enhanced dilation of the exercise-induced conduit artery diameter (brachial artery). However, this change did not in fact translate into increased blood flow during exercise, nor did it impact post-exercise vascular function (via FMD). However, when using repeat applications of IPC, Jeffries et al. studied the impact on metabolic and vascular adaptations. Following 7 days of repeated IPC the authors showed skeletal muscle oxidative capacity and microvascular muscle blood flow to be increased. These findings are consistent with enhanced mitochondrial and vascular function following repeated IPC and may be of clinical or sporting interest to enhance or offset reductions in muscle oxidative capacity.

Slysz and Burr were interested to see if the ergogenic efficacy of ischemic preconditioning (IPC) increased when combined with greater tissue level oxygen consumption and metabolite accumulation as a result of concurrent light intensity muscle contraction under IPC. Using participants for whom a traditional IPC stimulus was not effective, each underwent four experimental conditions in a cross-over experimental design: (i) no IPC control, (ii) traditional IPC, (iii) IPC with EMS, and (iv) IPC with treadmill walking. For conditions

where the IPC stress was magnified with the addition of muscle contractions while under occlusion, participants demonstrated a subsequent enhancement of the exercise performance response. These findings support the amplification of the ischemic preconditioning stimulus to augment the effect on exercise capacity.

Interested to see if IPC could help with recovery from repeated sprint exercise, Lopes et al. found that IPC did not change long-term heart rate recovery or heart rate variability throughout recovery, nor did IPC change any energy metabolism parameter. In conclusion, IPC accelerated short-term recovery to some extent, but did not change the long-term recovery of cardiac autonomic control from RSE, and such accelerator effect was not accompanied by any IPC effect on surrogates of energy metabolism responses to repeated sprint exercise.

Preconditioning may be performed with other modalities not just IPC. Thijssen et al. examined the impact of 12-week continuous training vs. high-intensity interval training on brachial artery endothelial ischaemia/reperfusion-injury in heart failure patients. They found that 12-week exercise training in heart failure patients mitigated endothelial ischaemia-reperfusion injury, an effect independent of the type of exercise. These changes may contribute to the cardioprotective effects of exercise training, whilst highlighting the potency of exercise as a pre-conditioning stimulus.

The opinion piece by Marocolo et al. listed some methodological concerns about protocol design, data analysis, and interpretation, of IPC research. They suggested the need for future studies to test shorter protocols (e.g., 2 × 2–3 min occlusion/reperfusion), which are more time-efficient (e.g., 8–12 min vs. 40 min) and more easily inserted in real-world settings of athletes/competitions if positive and meaningful findings are confirmed. Also, testing treatments controlled by different cuffing pressures (i.e., SHAM, IPC, and no cuff—control) should assess the effect of IPC on higher fitness subjects (i.e., elite athletes). Only then may we be able to draw robust conclusions as to whether IPC is suitable for recreational practitioners and/or elite athletes.

CONCLUSION

Taken on whole, the evidence produced from papers in this Research Topic highlight a number of potential roles for the use of blood flow manipulation to positively affect both health and human performance across the lifespan. While blood flow manipulation does appear safe and effective for increasing protein synthesis, muscle hypertrophy, and strength compared to non-restricted light-load exercise, the precise mechanisms of these changes still require characterization. Further optimization of these procedures with regard to the timing, pressure, periodization of use, and with regard to client/athlete training goals will help to guide its targeted and evidence-based use. The application of IPC appears to have the ability to alter exercise performance through pathways associated with local muscle perfusion and metabolism. Similar to BFR, the work herein sheds light on some potential mechanisms underlying the observed effects, but our ability to use these techniques in clinical or performance settings would benefit from further mechanistic work and an understanding of how these protocols are optimized when accounting for the interactions of participant characteristics, exercise parameters, and IPC stress. Future work should aim to address some of the limitations listed above for both BFR and IPC research as the current evidence in this Research Topic only sheds a limited light on the current area.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Conduit Artery Diameter During Exercise Is Enhanced After Local, but Not Remote, Ischemic Preconditioning

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Introduction: The ability of ischemic preconditioning (IPC) to enhance exercise capacity may be mediated through altering exercise-induced blood flow and/or vascular function. This study investigated the hypothesis that (local) IPC enhances exercise-induced blood flow responses and prevents decreases in vascular function following exercise.

Methods: Eighteen healthy, recreationally trained, male participants (mean ± *SD*: age 32 ± 8 years; BMI 24.2 ± 2.3; blood pressure 122 ± 10/72 ± 8 mmHg; resting HR 58 ± 9 beats min^{-1}) received IPC (220 mmHg; 4 × 5-min bilateral arms), REMOTE IPC (220 mmHg; 4 × 5-min bilateral legs), or SHAM (20 mmHg; 4 × 5-min bilateral arms) in a counterbalanced order prior to 30-min of submaximal (25% maximal voluntary contraction) unilateral rhythmic handgrip exercise. Brachial artery diameter and blood flow were assessed every 5-min throughout the 30-min submaximal exercise using high resolution ultrasonography. Pre- and post-exercise vascular function was measured using flow-mediated dilation (FMD).

Results: IPC resulted in enlarged brachial artery diameter during exercise [0.016 cm (0.003–0.03 cm), $P = 0.015$] compared to REMOTE IPC, but blood flow during exercise was similar between conditions ($P > 0.05$). Blood flow (l/min) increased throughout exercise (time: $P < 0.005$), but there was no main effect of condition ($P = 0.29$) or condition * time interaction ($P = 0.83$). Post-exercise FMD was similar between conditions ($P > 0.05$).

Conclusion: Our data show that local (but not remote) IPC, performed as a strategy prior to exercise, enhanced exercise-induced conduit artery diameter dilation, but these changes do not translate into increased blood flow during exercise nor impact post-exercise vascular function.

Keywords: ischemic preconditioning, cardiovascular, endothelial function, handgrip exercise, blood flow

INTRODUCTION

Ischemic preconditioning (IPC) is an intervention whereby three to four brief periods of ischemia, followed by tissue reperfusion, confer protection against subsequent ischemic insults (Murry et al., 1986). A single episode of IPC applied to both the exercising (IPC) and non-exercising limbs (REMOTE IPC) can enhance exercise performance (Bailey et al., 2012b; Barbosa et al., 2015), although the ability of IPC to positively impact performance based on the findings of a meta-analysis remains to be determined (Marocolo et al., 2015). To date, the mechanism underlying any ergogenic response remains speculative. It is hypothesized that IPC may induce alterations in skeletal muscle, via mitochondrial activation (Kido et al., 2015), and better maintenance of vascular function following exercise (Bailey et al., 2012a). Blood flow to exercising muscles is regarded as an important factor in determining the muscle's capacity to generate and perform muscle work (Joyner and Casey, 2014). An intervention capable of enhancing oxygen delivery and nutrient exchange within the working muscles could be vital for enhanced exercise performance. Additionally, the suggestion that (REMOTE)IPC can enhance high-intensity muscular performance, via delaying resistance to fatigue development in small muscle mass exercise (Barbosa et al., 2015) and augment in maximal-intensity larger muscle performance outcome (Kraus et al., 2015; Paradis-Deschênes et al., 2016) remains an interesting area of research. Regardless of performance outcome in these tasks, the mechanisms underpinning any potential performance changes currently remain elusive.

In an isolated exercise model (handgrip exercise), one previous study demonstrated that handgrip performance (time to exhaustion) was enhanced after REMOTE IPC, yet no change in blood flow occurred (Barbosa et al., 2015). The duration of handgrip exercise was likely too short to assess a steady-state exercise response on the vasculature. Nonetheless, some evidence suggests that REMOTE IPC can affect the dilator responses in the contralateral brachial artery (Enko et al., 2011). For example, Bailey et al. (2012a,b) found that REMOTE IPC negated the usual post-exercise reductions in brachial artery endothelial function, measured via the flow-mediated dilation (FMD) technique, after a 5-km time trial on a treadmill (Bailey et al., 2012a). A subsequent study by Horiuchi et al. (2015) used near-infrared spectroscopy (NIRS) to measure local limb oxygenation as an index of forearm blood flow at rest, light- [10% maximal voluntary contraction (MVC)], and moderate-intensity (25% MVC) handgrip exercise. They found a larger vasodilation during moderate-intensity exercise when the bout was preceded by IPC (Horiuchi et al., 2015). Taken together, there is accumulating evidence that IPC and REMOTE IPC have a direct role on the vasculature which could contribute to the ergogenic effects during exercise. However, no study has explored whether IPC could directly affect the exercise-induced changes in diameter, blood flow, and function, and whether these changes are different between IPC and REMOTE IPC, while using a SHAM condition to assess control responses.

The primary aim of this study was to examine the exercise-mediated changes in artery diameter and blood flow in response to IPC and REMOTE IPC in healthy individuals. We hypothesized that both IPC and REMOTE IPC would cause a diameter increase, accommodating a larger blood flow during exercise. A secondary aim of the study was to examine whether IPC and REMOTE IPC could prevent the usual decline in post-exercise vascular function compared to a SHAM condition. Finally, we examined whether (REMOTE)IPC is capable of enhancing MVC capacity when preceded by 30 min of submaximal exercise.

MATERIALS AND METHODS

Participants

Eighteen healthy, recreationally trained males (mean ± SD: age 32 ± 8 years; BMI 24.2 ± 2.3; blood pressure 122 ± 10/72 ± 8 mmHg; resting HR 58 ± 9 beats min^{-1}) were recruited and provided written informed consent in accordance with the Declaration of Helsinki. Physical Activity Readiness Questionnaires were administered to ensure no participant had any cardiovascular or metabolic health issues that would prevent participation. Participants refrained from exercise, and consumption of alcohol at least 24 h and caffeine 6 h prior to all laboratory visits. Participants were instructed to standardized food and drink prior to each trial and visited the laboratory at the same time (±1 h) for each trial to delimit the influence of circadian variation on outcome measures (Jones et al., 2010). Laboratory visits took place in a temperate environment (dry bulb temperature 22.9 ± 0.6°C; relative humidity 49.6 ± 6.7%; barometric pressure 758.4 ± 2.1 mmHg). The study was approved by the local Ethics Committee (Anti-Doping Lab Qatar, Doha, IRB F2016000128).

Experimental Design

Participants reported to the laboratory on three separate occasions, performing unilateral handgrip exercise that was preceded by either Local (IPC), Remote (REMOTE IPC), or SHAM condition (in a randomized order). Upon arrival, a resting FMD test was performed followed by either IPC, REMOTE IPC, or SHAM. Both IPC (upper arms) and REMOTE IPC (upper legs) consisted of four sets of 5 min cuff inflation of the limbs (220 mmHg) followed by 5 min reperfusion periods. SHAM consisted of four sets of 5 min cuff inflations at low pressure (20 mmHg) on the upper legs. Following a 20 min rest period, participants performed 30 min of rhythmic (30 contraction/relaxation cycles/min), submaximal handgrip exercise at 25% MVC. Brachial artery blood flow was monitored throughout the exercise bout. Participants rested for 90 s and then performed a 30 s "all out" MVC handgrip contraction. A post-exercise FMD test was then performed 3 min after the completion of the maximal contraction bout (**Figure 1**). Visits were separated by 4–7 days.

Assessing Maximum Voluntary Contraction

Participants attended the laboratory on their initial visit and performed short (3 s) maximal voluntary handgrip isometric

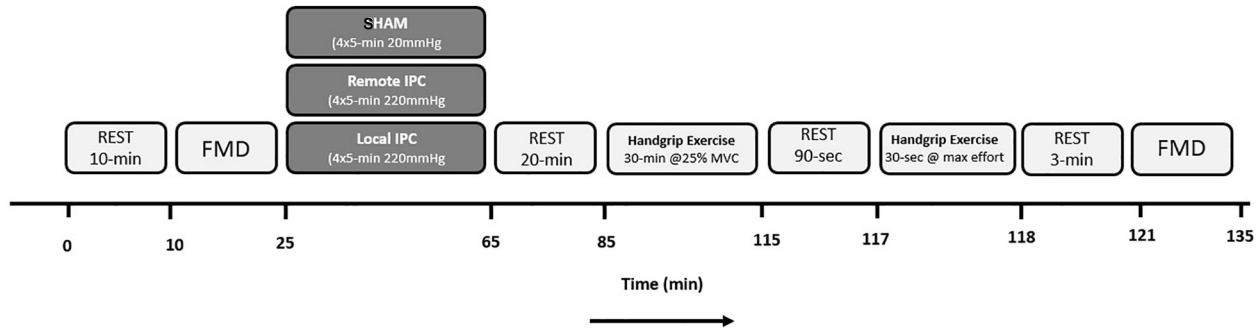

FIGURE 1 | Protocol schematic detailing experimental laboratory visits.

contractions (MVC); with each effort separated by 90 s rest. Each participant produced three efforts in total. A dynamometric handheld force transducer using MP35 hardware (Biopac Systems, Santa Barbara, CA, United States) and dedicated software (BSL Pro Version 3.6.7, Biopac Systems, Santa Barbara, CA, United States) was used to determine force generation. The maximum recorded value (kg) from these three efforts was used to determine MVC. For MVC determination, the signal was amplified (gain = 200) and recorded at a sampling frequency of 10 kHz. A standardized load of 20 kg (20 kg weight plate) was placed on the transducer 10 min after the system was turned on in order to calibrate the system prior to each trial.

Brachial Artery Endothelial Function

Following 5 min of supine rest, the participants arm was extended and positioned at an angle of approximately 80° from the torso. A cuff attached to a rapid inflator was placed distal to the olecranon process on the forearm. A 15 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (T3000: Terason, Burlington, MA, United States) was used to image the brachial artery in the distal third of the upper arm. Once a suitable image was obtained, the probe was held in position and settings were altered to optimize the longitudinal B-mode image of the lumen–arterial wall interface. Settings were identical between all FMD assessments. When collecting continuous Doppler velocity, the lowest insonation angle (<60°) was used. Following 1 min of baseline recording, the forearm cuff was inflated (>200 mmHg) for 5 min. Image recording commenced 30 s before cuff deflation and continued for 3 min thereafter (Black et al., 2008). Variables analyzed from FMD analysis that are displayed in **Table 3** consist of: peak diameter response (defined as the maximum brachial artery diameter obtained post-cuff release); time to peak diameter (defined as the time from cuff release to peak diameter response), and shear rate (four times velocity divided by diameter) area under the curve (SR_{AUC}; defined as the measured shear from the point of cuff release to peak dilation).

IPC Protocol

Ischemic preconditioning was performed in the supine position and cuff inflation pressure set at a standardized pressure (220 mmHg) in all experimental IPC conditions. With the use of a rapid inflator (E20) and air source (AG101) (Hokanson, Bellevue, WA, United States), 13.5 cm wide cuffs were inflated to 220 mmHg for 5 min, with the aim of preventing arterial inflow (Sharma et al., 2014). Subsequently, cuffs were deflated for 5 min, allowing reperfusion. This cycle was repeated four times in both IPC and REMOTE IPC conditions. A SHAM condition was also performed, in which cuffs were placed bilaterally on the upper thighs (4 × 5 min) and inflated to 20 mmHg. In each experimental trial, participants gave a perceived-discomfort rating following each IPC cycle. The discomfort rating was established using a numerical rating scale ranging from 0 (no discomfort) to 10 (maximum discomfort) (Ferreira-Valente et al., 2011) and is included for descriptive purposes (**Table 4**). A 20-min rest period was undertaken prior to experimental handgrip exercise performance. No participants were informed about the purpose or hypothesis of the study.

Handgrip Exercise

For all experimental trials, handgrip exercise was set at a submaximal intensity of 25% MVC. All sessions were performed at the same time of day, relative to the MVC visit in order to limit time of day effect on grip strength variation (Jasper et al., 2009). Participants remained in the supine position, with the dominant arm placed at an angle approximately 80° from the torso. Participants performed 30-min of rhythmic handgrip contractions on a dynamometric handheld force transducer using MP35 hardware (see the section "Assessing Maximum Voluntary Contraction" for details). The force scale was adjusted accordingly and the 25% MVC target intensity was clearly displayed on a screen in front of the participant. Real-time force (kg) feedback allowed participants to ensure they were working at the correct intensity for the duration of the trial. The rate of contractions was dictated by a metronome set at a rate of 30 contraction–relaxation cycles/minute.

Maximal Voluntary Contraction Task

Following cessation of rhythmic handgrip exercise, a 90-s recovery was allocated. Once rest time had elapsed, participants completed a 30-s forearm MVC using the same dynamometric

handheld force transducer previously mentioned. Both peak force (kg) and area under the curve (kg) were recorded.

Blood Flow Measurements

During the handgrip task, brachial artery diameter and blood velocity were measured with a linear array probe attached to a high-resolution ultrasound machine (T3000: Terason, Burlington, MA, United States). The probe was placed on the distal third of the upper arm for image consistency. Blood flow velocity (derived from Doppler region of interest at 30 Hz) was measured with an insonation angle <60°. Measurements were taken for 60 s per time point and mean values were calculated. Measures were performed at 1, 5, 10, 15, 20, 25, and 29 min during the 30-min handgrip task. Antegrade blood velocity was measured during the interval between contractions, while retrograde blood velocity occurred during muscular contraction.

Brachial Artery Diameter and Blood Flow Analysis

Custom-designed edge-detection software was used to analyze all recordings. This method allowed analysis to be performed largely independent of investigator bias. Blood flow (the product of lumen cross-sectional area and Doppler velocity) was determined from the synchronized diameter and velocity data at a sampling rate of 30 Hz. Shear rate (s^{-1}) (independent of viscosity) was calculated as four times mean blood velocity/vessel diameter. The semi-automated software, compared with manual methods, significantly reduces observer error and has shown previous intra-observer coefficients of variation (CoV) of 6.7% (Woodman et al., 2001). Intra-observer CoV in measurement of baseline arterial diameter in the current study was reported as 2.2 ± 1.4%. All files were analyzed by the same member of the research team (SC) to maximize reliability between trials. We also controlled for the baseline diameter measured before the introduction of hyperemia in each FMD test. This allometric approach is more accurate for scaling changes in diameter than simple percentage change, which makes implicit assumptions about the relationship between baseline diameter and peak diameter (Packard and Boardman, 2008; Atkinson and Batterham, 2013).

Statistical Analysis

All data were analyzed using linear mixed modeling. The primary outcome variable was blood flow response during exercise. This and all variables measured during exercise were analyzed with condition (three levels: IPC, REMOTE IPC, and SHAM) and time point (seven levels: minutes 1, 5, 10, 15, 20, 25, and 29 of exercise). Brachial artery endothelial function measurements were analyzed with condition (three levels: IPC, REMOTE IPC, and SHAM) and time-point (two levels: pre- and post-exercise). The least-significant method was employed for pairwise comparisons (Perneger, 1998). Data are presented in the text as the mean difference (95% CI). The level of significance (alpha) was set at $P = 0.05$. Any P-value that was reported as 0.00 in SPSS is reported in the current manuscript as $P < 0.005$.

RESULTS

Artery Responses During Exercise

Diameter

Diameter increased throughout the 30-min exercise bout ($P < 0.005$) (**Figure 2**). There was a main effect of condition ($P = 0.03$) whereby diameter during exercise following IPC was 0.016 cm (0.003–0.03) greater compared to REMOTE IPC ($P = 0.015$), while the difference with SHAM did not reach statistical significance [0.013 (−0.005 to 0.03) cm; $P = 0.16$]. Diameter changes between REMOTE IPC and SHAM [−0.03 (−0.16 to 0.10) cm] were not different ($P = 0.66$). There was no condition * time interaction effect ($P = 0.48$).

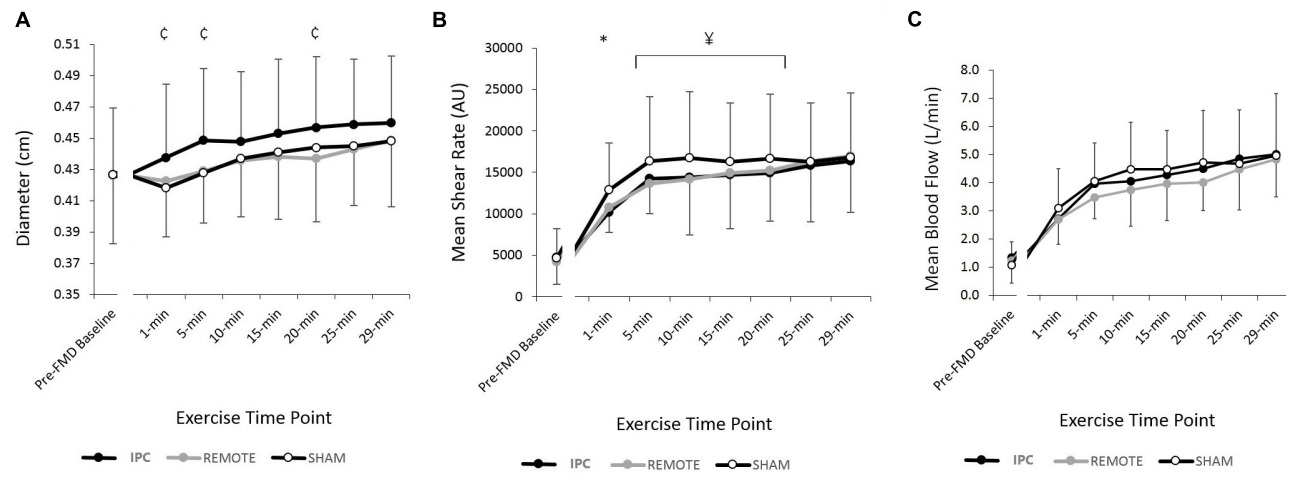

FIGURE 2 | Mean ± SD: data reported for **(A)** brachial artery diameter (cm) **(B)** shear rate (s^{-1}), and **(C)** blood flow (l/min) during 25% MVC handgrip exercise for each conditions. ¥ represents a significant difference between IPC and SHAM; ¢ represents a significant difference between IPC and REMOTE; and * represents a significant difference between IPC and both conditions.

Blood Velocity

There was a main effect of time ($P < 0.005$) and condition ($P < 0.005$) with both IPC [−2.6 (−3.9 to −1.4) cm/s; $P < 0.005$] and REMOTE IPC [−2.2 (−3.4 to −1) cm/s; $P = 0.001$] resulting in lower velocity *versus* SHAM. There was no difference between IPC and REMOTE IPC [−0.42 (−1.95 to 1.12) cm/s; $P = 0.89$]. There was no condition * time interaction ($P = 0.78$) (**Table 1**).

Blood Flow

Blood flow increased throughout exercise (time: $P < 0.005$), but there was no main effect of condition ($P = 0.29$) or condition * time interaction ($P = 0.83$) (**Figure 2**).

Shear Rate, Antegrade Shear, and Retrograde Shear

There was a main effect of time for mean shear rate (SR_{MEAN}) (**Figure 2**), antegrade shear (SR_{ANT}), and retrograde shear (SR_{RET}) (**Table 1**), with all three variables increasing with exercise duration (all $P < 0.005$, respectively). A main effect of condition was present for SR_{MEAN} ($P < 0.005$) with IPC being lower [−688 s^{-1} (−1369 to −6); $P = 0.048$] than REMOTE IPC and lower [−1756 s^{-1} (−2435 to −1078); $P < 0.005$] than SHAM. SR_{MEAN} for REMOTE IPC was −1069 s^{-1} (−1736 to −402) lower than SHAM ($P = 0.002$). There was no condition * time interaction ($P = 0.76$).

There was a significant effect of condition ($P < 0.005$) for SR_{ANT} (**Table 1**), with IPC resulting in −938 s^{-1} (−1648 to −227; $P = 0.01$) lower SR_{ANT} compared to REMOTE IPC and −2116 s^{-1} (−2823 to −1408; $P < 0.005$) lower SR_{ANT} compared to SHAM. REMOTE IPC resulted in −1178 s^{-1} (−1873 to −483; $P = 0.001$) lower SR_{ANT} when compared to SHAM. There was no condition * time interaction ($P = 0.89$). Additionally, no main effect of condition or condition * time interaction was evident in SR_{RET} ($P = 0.68$).

Brachial Artery Endothelial Function

A significant main effect of time was evident for brachial artery FMD (**Figure 3**). FMD decreased by 1.9% (−2.8, −1.03) from pre- to post-exercise ($P < 0.005$). There were no main effects of condition or condition * time interaction ($P > 0.05$, respectively). Results did not alter when the data were allometrically scaled for baseline diameter.

A significant main effect of time ($P < 0.005$) was evident for peak diameter (**Table 2**). Peak diameter increased by 0.03 (0.024–0.036) cm from pre- to post-exercise. There was evidence of a main effect of condition but this did not reach statistical significance ($P = 0.09$). Peak diameter was 0.008 (0.001–0.16) cm larger after IPC compared to SHAM ($P = 0.03$). There were negligible differences in peak diameter between IPC and REMOTE IPC [0.005 (−0.003 to 0.012) cm; $P = 0.21$] and also between REMOTE IPC and SHAM conditions [0.004 (−0.004 to 0.011) cm; $P = 0.35$]. There was no interaction between condition * time ($P = 0.14$).

A main effect of time was evident for baseline diameter (cm) (**Figure 3**), time to peak diameter (seconds), and shear rate under-the-curve (from cuff deflation to peak dilation; SR_{AUC}) (**Table 2**) which all increased from pre- to post-exercise ($P < 0.005$; **Table 3**). There was no main effect of condition

TABLE 1 | Mean ± SD reported for blood velocity (cm/s), antegrade shear (SR_{ANT}), and retrograde shear (SR_{RET}) for each time point during submaximal rhythmic handgrip exercise.

Variable	Condition	Time point								*P*-value
		Pre-exercise	1-min	5-min	10-min	15-min	20-min	25-min	29-min	condition
Velocity (cm/s)	IPC	9.21 ± 5.33	18.2 ± 5.8¥	25.8 ± 10	26.3 ± 9.1¥	27.1 ± 9	27.8 ± 9.4	29.7 ± 9.7	30.7 ± 13	
	REMOTE	8.28 ± 5.23	18.9 ± 5.2	24.1 ± 5.6#	25.3 ± 10.5	26.7 ± 10.7	27.1 ± 8.8	29.6 ± 11.6	31 ± 10.3	$P < 0.005$
	SHAM	7.93 ± 6.71	22.2 ± 6.7	28.5 ± 11.7	30 ± 12.5	29.5 ± 11.1	30.5 ± 12.7	29.8 ± 11.9	31.1 ± 13.2	
Antegrade	IPC	5323 ± 2390	11, 440 ± 4188*	15, 962 ± 7200¥	16, 089 ± 6113¥	16, 244 ± 6367¥	16, 478 ± 6248¥	17, 122 ± 6530	17, 608 ± 8131	
shear (SR_{ANT})	REMOTE	4850 ± 2466	12, 382 ± 2860ç	15, 576 ± 3980	16, 183 ± 6746	16, 674 ± 6695	16, 991 ± 6053	17, 931 ± 7314	18, 466 ± 6627	$P < 0.005$
	SHAM	4861 ± 3812	14, 419 ± 6164	18, 333 ± 8191	18, 620 ± 8225	18, 298 ± 7368	18, 519 ± 7970	18, 172 ± 7248	18, 493 ± 7932	
Retrograde	IPC	(−)588 ± 692	(−)1250 ± 709	(−)1718 ± 900	(−)1685 ± 853	(−)1535 ± 765	(−)1564 ± 834	(−)1315 ± 812¥	(−)1236 ± 787	
shear (SR_{RET})	REMOTE	(−)619 ± 624	(−)1633 ± 1020	(−)1983 ± 1171	(−)2036 ± 981	(−)1765 ± 962	(−)1771 ± 852	(−)1624 ± 885	(−)1536 ± 795	$P = 0.27$
	SHAM	(−)651 ± 695	(−)1563 ± 919	(−)2021 ± 1109	(−)1911 ± 1126	(−)2004 ± 1251	(−)1859 ± 1148	(−)1919 ± 1089	(−)1711 ± 1034	

*#represents a significant difference between REMOTE and SHAM; ¥represents a significant difference between IPC and SHAM; çrepresents a significant difference between IPC and REMOTE; and *represents a significant difference between IPC and both conditions.*

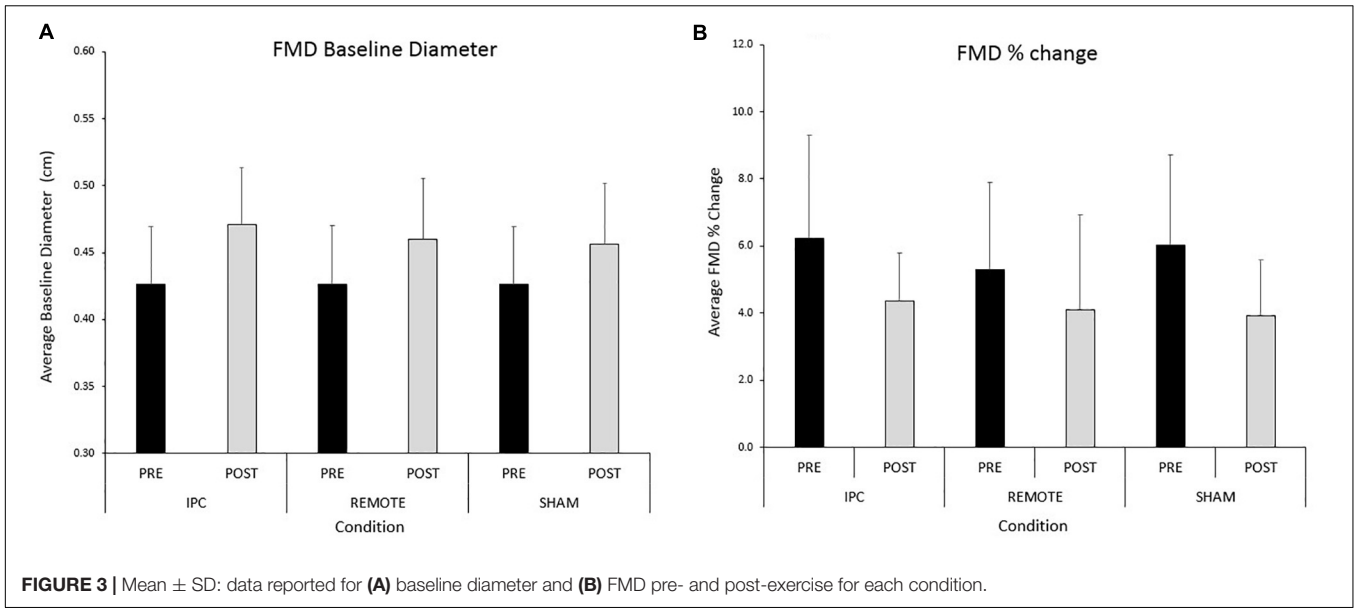

FIGURE 3 | Mean ± SD: data reported for **(A)** baseline diameter and **(B)** FMD pre- and post-exercise for each condition.

TABLE 2 | Pre- and post-exercise FMD responses (mean ± SD) for peak diameter (cm), time to peak dilation (s), and baseline shear rate (SR_{AUC}).

Condition	IPC		REMOTE		SHAM		*P*-Value
Time point	Pre	Post	Pre	Post	Pre	Post	Condition
Peak diameter response (cm)	0.45 ± 0.05	0.49 ± 0.05 ¥	0.45 ± 0.04	0.48 ± 0.04	0.45 ± 0.05	0.47 ± 0.05	$P = 0.09$
Time to peak (s)	65 ± 37	108 ± 42	62 ± 21	100 ± 33	78 ± 40	99 ± 31	$P = 0.71$
Response SR_{AUC}	19,038 ± 8724	42,642 ± 16585	16,678 ± 6983	36,176 ± 10,079	21,093 ± 8114	39,001 ± 12,777	$P = 0.18$

¥ *represents a significant difference between IPC and SHAM.*

TABLE 3 | Mean ± SD values for 30 s mean force production (kg) and peak (1 s) force production (kg), respectively, across conditions.

Condition	LOCAL	REMOTE	SHAM	*P*-Value
30 s force (kg)	20.9 ± 6.3	22.1 ± 6.2	21.5 ± 4.9	0.4
Peak force (kg)	25.3 ± 6.3	25.6 ± 6.7	25.6 ± 5.6	0.91

or condition * time interaction for any variable ($P > 0.05$, respectively).

Maximal Voluntary Contraction Task

No main effect of condition was evident for either peak force (kg), area under the curve (kg) during the 30-s MVC ($P > 0.05$, **Table 3**).

DISCUSSION

The aim of this study was to examine the exercise-mediated changes in artery diameter and blood flow pattern in response to IPC and REMOTE IPC in healthy individuals. We also examined whether IPC and REMOTE IPC could prevent the usual decline in post-exercise vascular function. The novel findings from the current study are (i) brachial artery diameter was greater during exercise following IPC when compared to REMOTE IPC, but this does not translate to a greater conduit artery blood flow between IPC and REMOTE IPC and (ii) neither IPC nor REMOTE IPC prevented the attenuation in brachial artery FMD following 30 min of hand grip exercise *versus* a SHAM condition. Taken together this data suggest that IPC impacts the vasculature more during exercise when compared to REMOTE IPC, leading to a larger diameter change for a given shear stress. However, neither protocol (IPC or REMOTE IPC) was found to enhance blood flow during exercise or prevent the drop in vascular function after exercise.

Hemodynamics During Handgrip Exercise

This is the first study to compare the effects of two different IPC protocols (local and REMOTE IPC) on exercise-mediated conduit artery diameter and blood flow. The current data show IPC resulted in a larger increase in conduit artery diameter that was maintained throughout exercise *versus* REMOTE IPC. While we did not perform a diameter measurement immediately prior to exercise, the larger diameter was evident in the first minute during exercise and continued to remain larger throughout exercise. Whether IPC exerted this dilatory response prior to the onset of exercise *versus* REMOTE IPC could not be determined

TABLE 4 | Mean ± SD values for perceived discomfort of ischemic preconditioning (IPC) and SHAM interventions.

	Perceived discomfort of condition (ratings 0–10)					
	Average	**0–10 min**	**10–20 min**	**20–30 min**	**30–40 min**	**Mean discomfort rating**
IPC	3.4 ± 0.3	3.8 ± 2	3.4 ± 1.9	3.3 ± 2.1	3.2 ± 1.9	Light to moderate
REMOTE	3.7 ± 0.5	4.4 ± 1.8	3.7 ± 1.9	3.4 ± 2	3.3 ± 1.9	Light to moderate
SHAM	0.2 ± 0	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5	No discomfort

in our design. The capacity of IPC to have direct and immediate effects on vasodilation of the conduit arteries infers that IPC could be a useful tool in improving arterial health. For example, in line with the ability of repeated exercise to enhance vascular function (Thijssen et al., 2010), regular episodes of IPC have similarly been shown to enhance arterial health (Jones et al., 2015). The observations in the present study provide further support for the potential benefits of IPC on vascular health.

Potential explanations for the larger diameter between IPC and REMOTE IPC include different impact of shear stress. Based on our observations, shear stress levels did not increase after IPC during exercise. While not measured in the current study, shear stress may have mediated larger nitric oxide release at the site of ischemia, or adenosine-mediated actions as a direct consequence of local IPC (Tapuria et al., 2008; Joyner and Casey, 2014), contributing to a larger diameter increase observed in the current study. When accounting for differences in mean shear rate during exercise, both IPC and REMOTE IPC resulted in lower blood velocity *versus* SHAM. It could be hypothesized that an ischemia-induced increase in diameter following IPC was responsible for producing lower velocity throughout exercise compared to SHAM; however, REMOTE IPC exerted similar responses in comparison, without significantly lower vasodilation response *versus* IPC. The underlying changes in blood velocity responses in both (REMOTE) IPC conditions, when compared to SHAM, are therefore unclear and may deserve further investigation.

An alternative explanation is that IPC affects the sympathetic nervous system (SNS), given that vascular tone is the product of the competitive balance between intrinsic local vasodilator function and adrenoceptor-mediated vasoconstriction. Possibly, IPC caused a reduction in sympathetic nerve activity (SNA), leading to a relative increase in artery diameter during exercise. Lower SNA has been observed following limb ischemia reperfusion injury preceded by IPC using the gold standard microneurography (Lambert et al., 2016). However, it has recently been reported that no change in SNA was present during static handgrip at 30% MVC, following IPC *versus* a SHAM condition (Incognito et al., 2017). Based on the available evidence, we cannot currently elucidate this mechanism. Given the larger diameter observed in the current study, however, we can state that IPC applied locally may exert a larger influence on the vasculature during exercise.

Despite the changes in diameter, this did not accommodate a larger blood flow during exercise. In fact, we even observed a lower mean and antegrade shear following IPC when compared to REMOTE IPC throughout the exercise. This was especially apparent during the first 20-min of exercise. Our findings are in line with a previous study, which found that REMOTE IPC (lower-limb) prior to handgrip exercise to exhaustion did not alter brachial artery blood flow (Barbosa et al., 2015). Nonetheless, this previous study found enhanced time-to-exhaustion after REMOTE IPC. Possibly, local redistribution of blood and/or changes in oxygen extraction (in the presence of preserved total blood flow to a limb) may explain the ergogenic effects observed in those studies. In support of this hypothesis, some studies have reported enhanced tissue oxygenation as an index of muscle perfusion capacity using NIRS when exercise is preceded by IPC (Paradis-Deschênes et al., 2016). Taken together, exercise-induced blood flow to the exercising limb unlikely increases after (REMOTE) IPC. Future work should explore whether local processes, contributing to redistribution of blood flow or oxygen uptake, may contribute to the potential ergogenic effects of (REMOTE) IPC, as currently it is unclear to an ischemia-induced enhancement in conduit artery vasodilation would serve benefit to exercise performance when applied in whole body performance models.

Brachial Artery Endothelial Function

Upon cessation of continuous exercise of a sufficient intensity, brachial artery FMD displays a biphasic response characterized by an immediate reduction and a return toward pre-exercise levels 1-h post-exercise (Birk et al., 2013). Previously, Bailey et al. (2012a) demonstrated that a bout of REMOTE IPC was capable of attenuating this immediate post-exercise reduction in FMD after a 5 km-TT on the treadmill. In the current study, neither IPC nor REMOTE IPC prevented the exercise-induced reduction in FMD *versus* SHAM exercise. There are a number of potential explanations for these contrasting findings. Firstly, handgrip exercise activates <1 kg of muscle *versus* larger muscle mass exercise such as running which can activate up to 10–15 kg of muscle (Rådegran et al., 1999; Joyner and Casey, 2014). Even during intense handgrip exercise, only ∼70% of the available delivered oxygen is extracted from arterial blood, suggesting the muscles in these modalities may be over-perfused when working in isolation (Joyner and Casey, 2014). While small muscle mass exercise is necessary to investigate the localized effects of exercise, the hemodynamic response to this exercise mode is independent of both neural and central regulatory changes that occur in whole body exercise (Thijssen et al., 2010). Therefore, differences in responses between small- and large-muscle mass exercise tasks when measuring post-exercise vascular function are plausible (Joyner and Casey, 2014; Atkinson et al., 2015). A second explanation relates to the intensity of exercise, where strenuous, whole body exercise is associated with instantaneous vascular injury, subsequently leading to reductions in vascular

function (Birk et al., 2013). The oxidative challenge as previously discussed is likely lessened in small muscle mass exercise (Joyner and Casey, 2014). Therefore, exercise tasks utilizing large muscle mass at high intensities maybe more insightful when examining the ability of (REMOTE) IPC to prevent post-exercise vascular dysfunction.

In the current study, we observed no changes in peak force (kg) or mean force production (AUC) following either REMOTE IPC or IPC *versus* SHAM. While studies (Paradis-Deschênes et al., 2016) that have employed exercise modalities utilizing larger muscle mass with similar shear rate patterns in blood flow (Thijssen et al., 2009) have demonstrated improved maximal isometric knee-extension performance *versus* a SHAM condition. Aiming to establish the reason as to why, in the current study, we found no difference in 30-s MVC performance between conditions would be speculative. Especially when compared to reports that IPC can augment 30-s maximal performances in larger muscle mass tasks (Kraus et al., 2015) remains unclear. As previously discussed, differences between large- and small-muscle mass high intensity performance are plausible.

When aiming to establish if an optimal dose–response to IPC exists, assessing the impact of both IPC and REMOTE IPC treatment to a standardized intervention may be useful. We previously observed that both IPC and REMOTE IPC protocols resulted in the same cycling time trial performance (Cocking et al., 2017). While no change in power output occurred between (REMOTE)IPC conditions, IPC resulted in lower oxygen uptake *versus* REMOTE IPC, indicative of IPC-induced metabolic alterations (Cocking et al., 2017). In the current study, IPC resulted in a greater brachial artery dilation that was maintained throughout the exercise bout, resulting in lower mean shear rate and antegrade shear when compared to REMOTE IPC.

Limitations

Firstly, although handgrip exercise provides capability to measure muscle blood flow accurately, the limited "active" muscle mass (<1 kg) likely provides a different management of vasodilation responses to exercise stimuli when compared to whole-body work. It may therefore not be possible to apply these results to larger active muscle mass areas when running or cycling for example. Secondly, the participation number of $N = 18$ was likely inadequate to be sure of detecting changes in blood flow. We would also like to state that due to the lack of a pre-exercise ultrasound measure, we cannot confirm whether the enlargement in brachial artery diameter observed was an exercise-induced response or indeed an ischemia-induced response. Additionally, it may have been that the MVC effort performed at the end-point of steady-state exercise altered the post-exercise vascular function response in the current study. Finally, the difficulty of truly blinding IPC interventions through use of a 20-mmHg SHAM condition produces the possibility that an expectation bias may occur.

In summary, we demonstrate for the first time that IPC performed as a strategy prior to exercise causes enhanced conduit artery vasodilation, maintained during exercise, when compared to REMOTE IPC. While these vascular adjustments do not translate into increase blood flow during exercise, the larger diameter mediated by IPC could indicate acute bouts of IPC contribute to improving the health of arteries.

ETHICS STATEMENT

The study was approved by the local Ethics Committee (Anti-Doping Lab Qatar – IRB F2016000128). All participants provided written informed consent in accordance with the Declaration of Helsinki. Physical Activity Readiness Questionnaires were administered to ensure no participant had any cardiovascular or metabolic health issues that would prevent participation.

AUTHOR CONTRIBUTIONS

HJ, DT, MW, SC, and NC developed the concept for this research project. SC performed all data collection and analysis. SC, HJ, and DT drafted and finalized the manuscript. MW, DG, NC, HJ, and DT significantly contributed to the drafts toward the final product and critically reviewed the manuscript. All authors (SC, MW, DG, HJ, DT, and NC) provided valuable comments throughout and insights throughout the process contributing to the final version of this manuscript. All authors (SC, MW, DG, HJ, DT, and NC) approved the final version of this manuscript.

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frontiers in Physiology

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Enhanced Local Skeletal Muscle Oxidative Capacity and Microvascular Blood Flow Following 7-Day Ischemic Preconditioning in Healthy Humans

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Ischemic preconditioning (IPC), which involves intermittent periods of ischemia followed by reperfusion, is an effective clinical intervention that reduces the risk of myocardial injury and confers ischemic tolerance to skeletal muscle. Repeated bouts of IPC have been shown to stimulate long-term changes vascular function, however, it is unclear what metabolic adaptations may occur locally in the muscle. Therefore, we investigated 7 days of bilateral lower limb IPC (4 × 5 min) above limb occlusion pressure (220 mmHg; n = 10), or sham (20 mmHg; n = 10), on local muscle oxidative capacity and microvascular blood flow. Oxidative capacity was measured using near-infrared spectroscopy (NIRS) during repeated short duration arterial occlusions (300 mmHg). Microvascular blood flow was assessed during the recovery from submaximal isometric plantar flexion exercises at 40 and 60% of maximal voluntary contraction (MVC). Following the intervention period, beyond the late phase of protection (72 h), muscle oxidative recovery kinetics were speeded by 13% (rate constant pre 2.89 ± 0.47 min^{-1} vs. post 3.32 ± 0.69 min^{-1}; $P < 0.05$) and resting muscle oxygen consumption ($m\dot{V}O_2$) was reduced by 16.4% (pre 0.39 ± 0.16%.s^{-1} vs. post 0.33 ± 0.14%.s^{-1}; $P < 0.05$). During exercise, changes in deoxygenated hemoglobin (HHb) from rest to steady state were reduced at 40 and 60% MVC (16 and 12%, respectively, $P < 0.05$) despite similar measures of total hemoglobin (tHb). At the cessation of exercise, the time constant for recovery in oxygenated hemoglobin (O_2Hb) was accelerated at 40 and 60% MVC (by 33 and 43%, respectively) suggesting enhanced reoxygenation in the muscle. No changes were reported for systemic measures of resting heart rate or blood pressure. In conclusion, repeated bouts of IPC over 7 consecutive days increased skeletal muscle oxidative capacity and microvascular muscle blood flow. These findings are consistent with enhanced mitochondrial and vascular function following repeated IPC and may be of clinical or sporting interest to enhance or offset reductions in muscle oxidative capacity.

Keywords: NIRS, blood flow restriction, exercise, ischemia, mitochondria

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INTRODUCTION

Ischemic preconditioning (IPC), first described by Murry et al. (1986), is a technique that intermittently occludes circulatory blood flow, interspersed by periods of tissue reperfusion. Applied in this way, local IPC protects the myocardium from tissue injury following ischemic insult (Murry et al., 1986). In addition, remote adaptations in distal tissues not directly exposed to the ischemic stimulus have also been observed (Hausenloy and Yellon, 2008). Since its early inception, IPC has been used for many different purposes and can be applied acutely or repeated across a number of days. Acute IPC has been shown to protect organs, including the myocardium (Murry et al., 1986), kidney (Lee and Emala, 2000), liver (Yoshizumi et al., 1998), and skeletal muscle (Gurke et al., 2000), from the damage caused by a subsequent prolonged ischemic event. In addition, systemic effects of IPC have also been reported to modulate parasympathetic nervous system activity (Enko et al., 2011). Furthermore, an acute application of IPC has beneficial effects on exercise performance (de Groot et al., 2010; Kido et al., 2015; Patterson et al., 2015; Tanaka et al., 2016). Protection conferred from an acute exposure of IPC has led investigators to examine whether repeated application may elicit dose-dependent protection. Indeed, repeated IPC has been shown to offer greater protection against cardiac ischemia, in a dose-dependent manor, in animal models (Wei et al., 2011; Yamaguchi et al., 2015). In humans, repeated IPC has been applied across 7 consecutive days (Jones et al., 2014), episodically across an 8-week period (Jones et al., 2015), and twice daily application for 300 days (Meng et al., 2012). Here clinical efficacy has been shown in patients displaying reduced stroke recurrence (Meng et al., 2012), improved wound healing in diabetics (Shaked et al., 2015), increased coronary flow reserve in heart failure patients (Kono et al., 2014), reductions in systolic and diastolic blood pressure (Madias, 2011; Jones et al., 2014), and improved vascular health (Kimura et al., 2007; Jones et al., 2014, 2015). However, the mechanisms by which IPC exerts protection are not well understood. Two phases of protection have been described were an early phase offers immediate protection which lasts several hours, linked to the release of mediators such a bradykinin and adenosine. A second window of protection, the late phase, follows approximately 24 h later lasting between 48 and 72 h and is dependent on the induction of protective proteins (Loukogeorgakis et al., 2005).

Acute application of IPC has also been shown to improve local skeletal muscle oxygenation during exercise (Saito et al., 2004; Kido et al., 2015; Patterson et al., 2015; Tanaka et al., 2016). For example, Patterson et al. (2015) demonstrated an improved maintenance of muscle oxygenation during repeated sprint cycling during the early phase of protection. Furthermore, IPC enhances muscle deoxygenation dynamics during whole-body (Kido et al., 2015) and local muscular endurance exercise (Tanaka et al., 2016), suggestive of lower O_2 extraction and/or greater blood flow to skeletal muscle. In skeletal muscle, local application of IPC confers ischemic tolerance to subsequent ischemic events (Gurke et al., 2000). There is also evidence that IPC can protect against reductions in ischemic-induced glycogen depletion (Lintz et al., 2013), restore mitochondrial dysfunction (Thaveau et al., 2007; Mansour et al., 2012) and remotely lower energy metabolism during sustained ischemia (Addison et al., 2003), thus demonstrating a range of potential mechanisms whereby skeletal muscle function is enhanced. However, to date, little is known about the oxidative potential of skeletal muscle following repeated bouts of IPC.

The energetic demands of muscular work are largely supported by oxidative metabolism. Using near-infrared spectroscopy (NIRS), muscle oxidative capacity is reduced in clinical patient groups where normal muscle function is impaired, such as in motor-complete spinal cord injuries (Erickson et al., 2013), peripheral vascular disorders (Cheatle et al., 1991), or those with sedentary lifestyles (Brizendine et al., 2013). In comparison, endurance trained athletes display 5-fold increases in muscle oxidative function (Brizendine et al., 2013; Adami and Rossiter, 2017). Using NIRS alongside a series of transient arterial occlusions has enabled non-invasive reporting of muscle oxidative capacity with good reproducibility when compared to gold standard techniques such as magnetic resonance spectroscopy (MRS) (Ryan et al., 2013c) and *in situ* measures of respiratory capacity via muscle biopsy analysis (Ryan et al., 2014). Interventions that can stimulate adaptations in skeletal muscle function, other than physical exercise, may not only be useful for athletic populations but could improve or sustain physical activity in clinical populations, or facilitate recovery following brief periods of immobility. The reported increases in vascular conductance following repeated IPC (Jones et al., 2014), alongside demonstrable effects of acute IPC on exercise performance and local muscle O_2 dynamics (Kido et al., 2015; Patterson et al., 2015), led us to hypothesize that repeated bouts of IPC may stimulate a sustained enhancement of skeletal muscle oxidative capacity and microvascular blood flow. Therefore, we examined the effects of 7 consecutive days of bilateral lower-limb IPC on local skeletal muscle oxidative capacity and microvascular blood flow in healthy, young men.

MATERIALS AND METHODS

Participants

A total of 20 adult male participants volunteered for this study (age, 26 ± 5 years; stature, 180 ± 6 cm; body mass 80 ± 12 kg). The participants were all actively engaged in regular exercise (250 ± 150 min/week) that consisted of endurance running or where involved in team sports. They were non-smokers and not taking any medications. Participants were advised to maintain a normal training schedule throughout the IPC or sham intervention but not to undertake any additional training or to reduce their current training load. *A priori* sample size was calculated using G*Power (Version 3.1.9.3). This was determined according to changes in the deoxygenated hemoglobin/myoglobin (HHb) time course for recovery from moderate-intensity exercise following acute application of IPC [Δtime constant (Tc) = 4.6 s; *SD* = 1.7] (Kido et al., 2015). A total of 6 participants per group were deemed sufficient to

yield a power of 0.80 and $\alpha = 0.05$. A total of 10 participants were recruited to account for experimental mortality throughout the duration of the trial. Ethical approval was provided by the St. Mary's University Ethics Committee which was conducted in accordance with the 1964 Helsinki Declaration. All participants provided written, informed consent before testing.

Experimental Design

Participants first visited the laboratory to provide informed consent, conduct a maximal voluntary plantar flexion contraction (MVC) for determination of subsequent exercise intensities and for the purposes of allocating groups. Participants were also familiarized with the IPC pressure and electrical stimulation procedures used in the experimental trials. The main experimental trial began 48–72 h later after which participants attended the laboratory on nine occasions, across 11 consecutive days. All trials were conducted at the same time of day to eliminate circadian variation. On the first experimental visit, baseline measurements of muscle oxygenation were performed, followed by a series of arterial occlusions to establish resting muscle oxygen consumption ($m\dot{V}O_2$) and the rate of recovery from a brief electrical stimulation. An exercise protocol involving plantar flexion isometric contractions over a range of submaximal exercise intensities was performed on a dynamometer. Participants were ranked based on their MVC performance during the first visit and separated into an intervention (IPC) or sham (control) group using A-B-B-A. Each group then visited the laboratory on 7 consecutive days to perform an IPC or sham procedure. After the final intervention, participants returned to the laboratory 72 h later to replicate the tests performed on the first experimental visit. The 72-h time period was chosen as this was outside the reported early and late phases of protection conferred by IPC (Loukogeorgakis et al., 2005) and therefore would reflect a sustained adaptation to the IPC stimulus. Participants were instructed to avoid consumption of alcohol or caffeinated products for 24 h before, and strenuous exercise 48 h before, the baseline and post-intervention test.

Measures of $m\dot{V}O_2$

Following 10 min of supine rest on a padded table with both legs fully extended, a triangular pillow was placed under the right thigh and support placed under the participant's right ankle to optimize the lower leg position perpendicular to the floor (**Figure 1**). Participants were informed to not move their limbs throughout the testing procedures. A near-infrared spectroscopy (NIRS) optode (Portamon, Artinis medical systems) was placed on the gastrocnemius medialis (2/3 distance from the calcaneus and anterior fossa) and secured with an elastic bandage (Tiger Tear, Hampshire, United Kingdom) to prevent movement and covered with an optically dense black material to minimize the intrusion of extraneous light. The position of the probe was marked with indelible ink which was reapplied at regular intervals during the intervention protocol to ensure correct placement of the optode during the post-intervention trial. Two square electrodes (50 × 50 mm), attached to a neuromuscular stimulator (NeuroTrac Sports, Verity Medical LTD, Hampshire, United Kingdom), were placed immediately next to the NIRS optode at proximal and distal skin sites. A rapid inflating blood pressure cuff (width: 15 cm; Hokanson SC12D, Bellevue, WA, United States) in conjunction with a rapid cuff inflator (E20, Hokanson, Bellevue, WA, United States) supplied by an air compressor, was placed around the upper thigh.

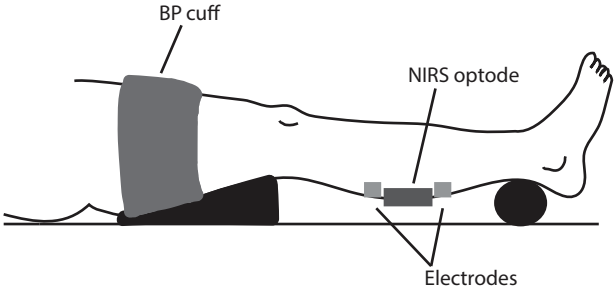

FIGURE 1 | Experimental setup.

Resting $m\dot{V}O_2$ was assessed by rapidly inflating the blood pressure cuff to 300 mmHg, ensuring full arterial and venous occlusion of the lower limb for 30 s. This procedure was then repeated after 3-min rest, with data averaged over the two measures. Following a further 3-min rest, the main test protocol began. To examine muscle oxidative recovery, twitch electrical stimulation (pulse duration 250 μS) was administered at a frequency of 6 Hz (Ryan et al., 2013a) for 15 s to increase muscle metabolic rate. Electromyostimulation allows non-selective and synchronous recruitment of muscle fibers in the region of interest (Gregory and Bickel, 2005). Current intensity was gradually increased over an initial 10 s to 60 mA for patient comfort (Doucet et al., 2012). The chosen current intensity elicited a twitch contraction across all individuals tested. It has previously been noted that small differences in stimulation do not influence measurements of $m\dot{V}O_2$ recovery kinetics (Ryan et al., 2013a). Immediately following the stimulation, a series of brief arterial occlusions (300 mmHg) were performed using the following protocol: cuff 1–5 = 5 s ON, 5 s OFF; cuff 6–10 = 5 s ON, 10 s OFF; cuff 11–15 = 10 s ON, 20 s OFF (Ryan et al., 2012). This protocol was used to characterize the recovery of $m\dot{V}O_2$ and has been shown to be reliable and reproducible when compared to phosphorus magnetic resonance spectroscopy (P-MRS) indexes of skeletal muscle oxidative function (Ryan et al., 2013c), and *in situ* high-resolution respirometry measures of mitochondrial respiratory capacity via muscle biopsy analysis (Ryan et al., 2014). The exercise/cuff protocol was performed twice with a 5-min rest period between protocols and the two tests were then averaged. To normalize the NIRS signal, an ischemia/hyperemia procedure was performed as follows. Following a brief 5-s electrical stimulation at 6 Hz, the cuff was inflated to 300 mmHg for 5 min (or until a plateau was reached). This represented maximal deoxygenation (0%) of the tissue under the optode. Following release of the cuff, a peak hyperemic response (representing 100% oxygenation) was recorded. The

short duration stimulation helped to minimize the duration of the ischemic cuff and avoid unnecessary discomfort. All data were normalized within each trial to this 'physiological' range to allow comparisons between individuals with differing ATT (Van Beekvelt et al., 2001; Ryan et al., 2012). Representative experimental data from a single individual trial is shown (**Figure 2**).

Calculation of $m\dot{V}O_2$

A blood volume correction factor was applied to each data point as previously described (Ryan et al., 2012) based on the assumption that during an arterial occlusion, the area under the probe is a closed system and therefore changes in oxygenated hemoglobin/myoglobin (O_2Hb) and deoxygenated hemoglobin/myoglobin (HHb) should occur in a 1:1 ratio. Briefly, a blood volume correction factor was calculated according to Eq. 1:

$$\beta(t) = \frac{[O_2Hb(t)]}{(O_2Hb) + [HHb(t)]} \quad (1)$$

where, β is the blood volume correction factor, t represents time, O_2Hb is the oxygenated hemoglobin/myoglobin signal and HHb is the deoxygenated hemoglobin/myoglobin signal. The blood volume correction factor was calculated for each data point and then applied to the raw NIRS data for O_2Hb (Eq. 2) and HHb (Eq. 3) as below:

$$O_2Hb_c(t) = O_2Hb(t) - [tHb(t) \times (1 - \beta)] \quad (2)$$

$$HHb_c(t) = HHb(t) - [tHb(t) \times \beta] \quad (3)$$

where, O_2Hb_c and HHb_c are the corrected oxygenated and deoxygenated hemoglobin/myoglobin signals, tHb is the relative total hemoglobin concentration from the NIRS device, β is the blood volume correction factor, and t is time. These values for O_2Hb_c and HHb_c thus represent the corrected data minus any blood volume changes.

The $m\dot{V}O_2$ was calculated by the slope of change in the corrected O_2Hb and HHb during the first 3 s of an arterial occlusion by using simple linear regression. We calculated a test–re-test reliability of 6.7% coefficient of variation (CV) for resting $m\dot{V}O_2$.

Following an electrically stimulated increase in metabolic rate, the repeated $m\dot{V}O_2$ measurements were then fitted to a monoexponential curve to derive a rate constant (k). This method utilizes linear modeling to report changes in $m\dot{V}O_2$ as an index of oxidative capacity (Hamaoka et al., 1996), according to Eq. 4:

$$y = End - Delta \times (1 - e^{-kt}) \quad (4)$$

where y is the relative $m\dot{V}O_2$ during arterial occlusion, End is the $m\dot{V}O_2$ immediately following the cessation of the electrical stimulation, Delta is the change in $m\dot{V}O_2$ from rest to end exercise, t is time, and k is the rate constant. The recovery of $m\dot{V}O_2$ to resting levels, therefore, represents the maximal oxidative capacity of the region of interest monitored in skeletal muscle. We calculated a test–re-test reliability for the k assessment of oxidative function using NIRS as 8.7% CV, which was comparable to that reported elsewhere for the same technique (Adami and Rossiter, 2017).

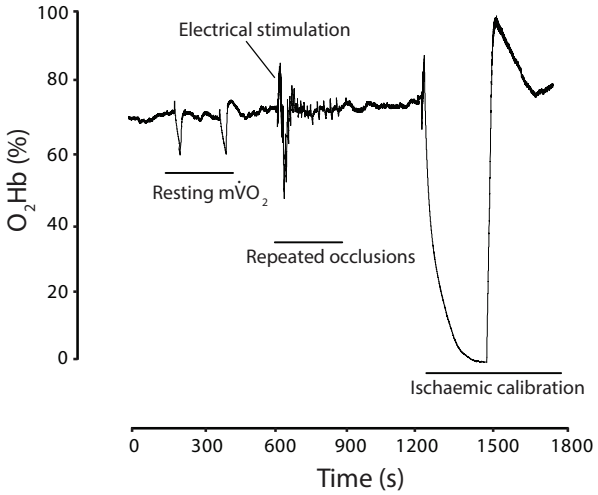

FIGURE 2 | Experimental trial illustrated with muscle oxygenated hemoglobin (O_2Hb) expressed as a percentage of an ischemic normalization. Two resting 30-s occlusions were performed to establish resting $m\dot{V}O_2$, followed by a 15-s electrical stimulation and a series of 15 brief occlusions. This was repeated but not shown for clarity. Finally, a 5-min ischemic normalization procedure was performed.

Submaximal Plantar Flexion Exercise

A total of 14 participants from the initial study (age, 26 ± 5 years; stature, 182 ± 5 cm; body mass 84 ± 12 kg) took part in an exercise protocol to further identify changes in local muscle metabolism and blood flow. Seven participants per group were recruited to complete this follow-up investigation due to availability of laboratory and the duration of the expanded testing protocol required. Participants (IPC n = 7; Sham n = 7) conducted a range submaximal isometric plantar flexion exercise tasks following tests of muscle oxidative capacity. They were seated upright on a dynamometer [Kin Com Auto Positioning (125 AP), Chattanooga Group Inc., Hixson, TN, United States] with the knee fully extended (0°) to ensure that the gastrocnemius contributed significantly to plantar flexor joint movement (Cresswell et al., 1995) and the hip joint was extended (124 ± 4°), as assessed using a goniometer. The foot was fixed in a neutral anatomical position, with the sole of the foot fixed perpendicular (90°) to the tibia. The lateral malleolus was visually aligned with the center of rotation of the dynamometer lever arm and the foot was securely fixed to the dynamometer foot plate with Velcro straps. All measures for each participant were recorded for future trials. Participants conducted a warm-up, comprising of 3 × 5-s submaximal isometric plantar flexion contractions at 25, 50, and 75% of perceived maximum effort, separated by 30-s rest. Maximal voluntary plantar flexion isometric contraction (MVC) was first established during the familiarization session to enable determination of a range of working submaximal exercise

intensities for pre and post exercise testing. Participants were instructed to perform three maximal isometric plantar flexion contractions and gradually develop force from rest to maximum over a 3- to 5-s time period, separated by 60-s rest. The peak plantar flexion torque associated with maximal voluntary contraction (MVC) was then recorded. If the 3rd contraction continued to increase, additional attempts were made until a plateau was reached.

During experimental visits participants performed intermittent plantar flexion contractions at 40, and 60% of MVC. The exercise protocol involved rhythmic isometric contractions in an equal work to rest ratio (2.5-s contraction: 2.5-s rest) for a total of 2 min. The pattern was maintained by following a digital metronome (total of 24 contractions). This exercise time was chosen as it elicited steady-state HHb in pilot trials. A feedback display of the actual force output was provided to the participants who matched it at the prescribed intensity. Recovery between the different intensity bouts was 3 min, or until NIRS data returned to baseline. Values for HHb and tHb were reported as the delta from baseline (30 s average prior to test) to steady state exercise to examine the metabolic demands of the exercise bout. The HHb signal which is regarded as blood volume insensitive during exercise was therefore used to indicate intramuscular oxygenation status (De Blasi et al., 1994). Following the cessation of exercise, the time course of recovery from exercise was modeled on O_2Hb off-kinetics to evaluate oxygen delivery and utilization (McCully et al., 1994b). A mono-exponential (Eq. 4) was fitted to the first point greater than 1 SD above the end exercise mean over 120 s and a Tc calculated.

NIRS Device

The NIRS signals were obtained using a portable unit consisting of 3 channels (Portamon, Artinis Medical Systems, Zetten, Netherlands). The system is a two-wavelength continuous wave system that simultaneously uses the modified Beer-Lambert law and spatially resolved spectroscopy methods. Changes in tissue O_2Hb, HHb, and tHb were measured using the differences in absorption characteristics of infrared light at 760 and 850 nm. Differential path factor (DPF) of 4 was used throughout. NIRS data was connected to a computer by Bluetooth for acquisition at 10 Hz.

IPC Protocol

For the IPC protocol, automatic inflation cuffs (14.5 cm width – Delfi Medical Innovations, Vancouver, BC, Canada) were placed on the proximal portion of both thighs. The inflatable cuffs were connected to a pressure gauge and were automatically inflated to 220 mmHg (IPC) to ensure maximum occlusion across all participants (de Groot et al., 2010). The sham group experienced a lower pressure (20 mmHg) using the same automatic inflation cuffs. The protocol involved 5-min occlusion, followed by 5-min reperfusion, which was repeated four times (lasting 40 min) in the supine position. This procedure was repeated for 7 consecutive days. To ensure the complete occlusion of arterial inflow to the limb, each individual had their limb occlusion pressure (LOP) assessed using a Doppler probe (UltraTec PD1, Ultrasound Technologies, Caldicot, United Kingdom) and automatic inflation cuff, as previously described (Loenneke et al., 2012). Average LOP for the IPC group was 140 ± 8 mmHg and for the sham group 151 ± 17 mmHg.

ATT, Heart Rate, and Blood Pressure

Adipose tissue thickness (ATT) was measured at the site of the NIRS optode, in duplicate, to the nearest 0.1 mm using skinfold calipers (Harpenden, Burgess Hill, United Kingdom). The average value of skin and subcutaneous tissue thickness for the IPC group was 6.9 ± 2.6 mm and for the sham group 6.4 ± 2.5 mm (range of 3.8–12.4 mm). This was less than half the distance between source and the detector (35 mm). There was no correlation between $m\dot{V}O_2$ and ATT when expressed as a percentage of the ischemic normalization (R^2 = 0.19437; $P > 0.05$). Blood pressure was monitored using an upper arm blood pressure monitor (Omron i-C10, Omron Healthcare Co., Kyoto, Japan) at the beginning of both experimental sessions, following 10 min of supine rest. Mean arterial pressure (MAP) was reported based on calculations of systolic blood pressure (SBP) and diastolic blood pressure (DBP): MAP = DBP + 0.33 (SBP − DBP). Following duplicate measures of oxidative capacity, heart rate responses were recorded in the supine position (Polar V800, Polar Electro OY, Kempele, Finland) for 2 min (pre-occlusion), 5 min (ischemic-normalization arterial occlusion), and 5 min (reperfusion). The periods were selected to obtain measurements of heart rate and heart rate variability (HRV) during rest and in response to acute lower-limb ischemia. The raw RR intervals were converted into beat-to-beat heart rate, after which potential ectopy and artifact was determined by a computer algorithm that identified all RR intervals outside the upper and lower limits (±25%) of a 5-beat moving average. Two time-domain measures of short-term HRV were determined: the standard deviation of all RR intervals (SDNN) and the square root of mean squared differences of successive normal RR intervals (RMSSD). Data were reported in milliseconds (ms). Conducting short-term HRV over = 2-min resting intervals has been commonly used in the literature (Dekker et al., 1997; Levy et al., 1998) as an indication of training adaptation or health status. The device used to measure HRV has been validated against ECG recordings (Giles et al., 2016). Room temperature and lighting was controlled throughout all trials (19.7 ± 0.5°C) and the participants were not given any instructions relating to their breathing patterns while measurements were taking place. The participants did not talk, consume food or drink, nor were they given any additional verbal or visual stimuli during the trial.

Statistical Analysis

Data are presented as means ± SD. After checks for normality, analysis of covariance (ANCOVA) was used to determine the difference between resting $m\dot{V}O_2$ and recovery kinetics, exercise intensity and recovery, heart rate, mean arterial pressure, and heart rate variability measurements. There was one independent variable with two levels (IPC vs. sham), with the participants' pre-test baseline data used as a covariate. Magnitude of effects was calculated with partial eta-squared

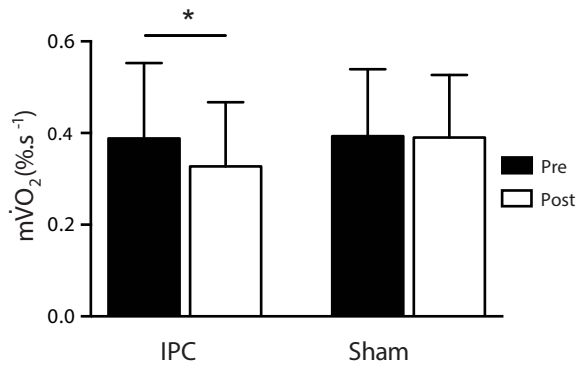

FIGURE 3 | Resting muscle oxygen consumption pre (black) and post (white) for the IPC (n = 10) and sham (n = 10) groups. *$P > 0.05$.

(η_p^2) according to the following criteria: 0.02, a small difference; 0.13, a moderate difference; 0.26, a large difference (Cohen, 1988). Statistical analysis was performed using SPSS 21 (IBM, Armonk, NY, United States). Curve fitting and display of data was performed with GraphPad Prism (GraphPad Software, La Jolla, CA, United States). Statistical significance was set at $P < 0.05$.

RESULTS

Resting Muscle Oxygen Consumption

Resting $m\dot{V}O_2$ was comparable at baseline between groups (IPC 0.39 ± 0.16%.s^{-1}, Sham 0.39 ± 0.15%.s^{-1}; $P > 0.05$). Following the 7-day IPC intervention, $m\dot{V}O_2$ was reduced by 16.4% (IPC 0.33 ± 0.14%.s^{-1}) compared to no change in the control group (Sham 0.39 ± 0.14%.s^{-1}) [$F_{(1,0.018)} = 4.493$, $P = 0.039$, $\eta_p^2 = 0.21$], indicating a moderate effect (**Figure 3**).

Muscle Oxidative Capacity

Immediately following a 15-s electrical stimulation during pre-intervention testing, end contraction $m\dot{V}O_2$ was increased relative to resting $m\dot{V}O_2$ in both conditions (IPC 0.39 ± 0.16 to 5.01 ± 0.92%.s^{-1}; Sham 0.39 ± 0.15 to 4.09 ± 0.94%.s^{-1}). Following the 7-day intervention procedure, there were no significant changes in end contraction $m\dot{V}O_2$, however, η_p^2 indicated a moderate effect with a trend toward a ∼13% reduction in the IPC group (IPC pre 5.01 ± 0.92 to post 4.36 ± 0.38%.s^{-1}) and minimal changes in the sham group (∼1.7%) (Sham pre 4.09 ± 0.94 to post 4.01 ± 0.68%.s^{-1}) ($P > 0.05$, $\eta_p^2 = 0.13$). To model the recovery from 15-s electrically stimulated muscle contractions, the rate constant (k) for the recovery of $m\dot{V}O_2$ was assessed via a series of brief occlusions prior to the intervention procedure (Baseline IPC 2.89 ± 0.47 min^{-1}; Sham 3.18 ± 1.36 min^{-1}). The 72 h following 7-day IPC, the rate constant for $m\dot{V}O_2$ recovery was increased ∼13%, with no change in the control group (< 3%) (IPC pre 2.89 ± 0.47 to post 3.32 ± 0.69 min^{-1}; Sham pre 3.18 ± 1.36 to post 3.25 ± 1.50 min^{-1}) [$F_{(1,19)} = 4.897$, $P = 0.041$, $\eta_p^2 = 0.224$], indicating a moderate effect (**Figures 4A,B**). A total of 9 out of the 10 participants in the IPC group demonstrated enhanced oxidative capacity (IPC range: 2–50%), with minor changes in the control group (Sham range: < 7%).

Muscle Oxygenation Responses to Submaximal Exercise

The IPC reduced delta HHb at exercise intensities of 40% MVC (IPC 16%; Sham 2% [$F_{(1,13)} = 8.867$; $P = 0.014$; $\eta_p^2 = 0.470$]) and 60% MVC (IPC 12%; Sham 3% [$F_{(1,13)} = 4.217$; $P = 0.05$; $\eta_p^2 = 0.277$]) with no change in sham (**Table 1**). In contrast, tHb was not different between condition ($P > 0.05$) (**Table 1**). The Tc for recovery following exercise was increased by ∼33 and 43% at 40 and 60% of MVC, respectively [40% MVC $F_{(1,13)} = 0.824$; $P = 0.038$; $\eta_p^2 = 0.46$] [60% MVC $F_{(1,13)} = 6.742$; $P = 0.025$; $\eta_p^2 = 0.38$], with no changes in the sham group (**Figure 5B**). A representative figure illustrates the shift in reoxygenation after exercise at 40% MVC following 7-day IPC (**Figure 5A**).

Cardiovascular Adaptations

Mean arterial pressure (MAP) was not different between groups ($P = 0.578$; $\eta_p^2 = 0.019$) (**Table 2**). There was no difference between IPC and sham groups for post-trial measures of 2-min resting heart rate ($P = 0.255$; $\eta_p^2 = 0.085$), 2-min RMSSD ($P = 0.110$; $\eta_p^2 = 0.162$), 2-min SDNN ($P = 0.076$; $\eta_p^2 = 0.195$), 5-min occlusion heart rate ($P = 0.531$; $\eta_p^2 = 0.027$), and 5-min recovery heart rate ($P = 0.867$; $\eta_p^2 = 0.002$) (**Table 2**).

DISCUSSION

This study provides the first evidence that skeletal muscle oxidative function is enhanced following repeated IPC. We report a number of important observations. First, repeated bouts of bilateral lower-limb IPC across 7 consecutive days increased skeletal muscle oxidative capacity. Second, we observed a decrease in resting skeletal muscle metabolism. Third, there was evidence of enhanced microvascular oxygenated blood flow following IPC that facilitated a rapid recovery from submaximal exercise. Finally, systemic measures of resting blood pressure, heart rate and autonomic function did not change following IPC. Together, these findings suggest that local adaptations, distal to the IPC stimulus, potentiate oxidative function, and microvascular blood flow beyond the late phase (24–72 h) of protection, despite no apparent systemic cardiovascular changes.

Enhanced Local Skeletal Muscle Oxidative Capacity

A ∼13% increase in skeletal muscle oxidative function was achieved in this study, by administering four bouts of arterial occlusion for 5 min with a time equivalent reperfusion, over a 7-day period. The participant group used for this investigation were healthy, young (average ∼26 years), active males (average engagement in exercise ∼4 h/week), with baseline measures of oxidative function that support a good level of physical fitness (∼2.89 min^{-1}) when compared values reported elsewhere using

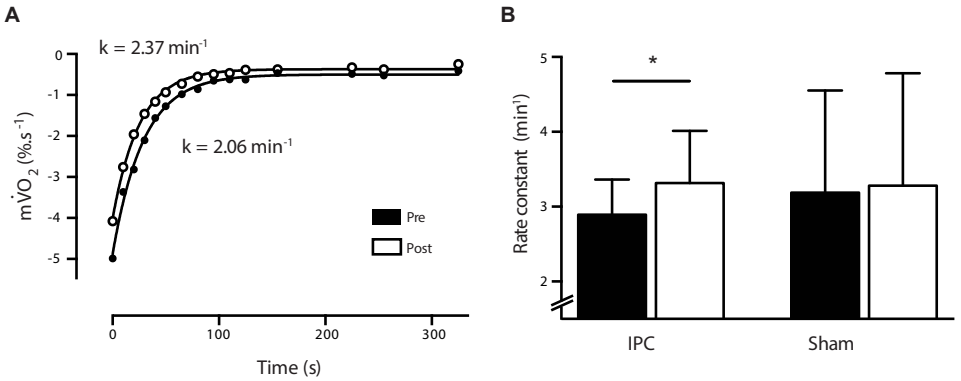

FIGURE 4 | (A) Representative (n = 1) NIRS $m\dot{V}O_2$ recovery curve from pre (white circle) and post (black circle) following a 7-day IPC intervention. Data is fitted to a non-linear monoexponential curve and a rate constant (k) derived. **(B)** Bar graph illustrates average rate constants for pre (black) and post (white) for the IPC (n = 10) and sham (n = 10) groups. *$P > 0.05$.

TABLE 1 | Delta changes in HHb and tHb following submaximal intermittent plantar flexion contractions at 40 and 60% of 1RM among IPC (n = 7) and sham (n = 7) groups.

	(HHb) delta (a.u.)		(tHb) delta (a.u)	
	IPC Pre	**IPC Post**	**IPC Pre**	**IPC Post**
40% MVC	26.08 ± 5.81	21.96 ± 6.49*	55.81 ± 8.77	54.39 ± 8.80
60% MVC	29.06 ± 7.59	25.62 ± 6.79*	66.01 ± 7.68	64.60 ± 13.77
	Sham Pre	**Sham Post**	**Sham Pre**	**Sham Post**
40% MVC	25.62 ± 11.26	26.05 ± 6.96	43.96 ± 15.84	45.97 ± 8.87
60% MVC	25.88 ± 9.92	27.76 ± 9.53	50.08 ± 16.99	51.65 ± 9.76

*Values are presented as mean ± SD. HHb, deoxygenated hemoglobin; tHb, total hemoglobin; MVC, maximal voluntary contraction; a.u., auxillary units; *$P < 0.05$ comparing pre to post.*

this NIRS technique. For example, elite athletic populations (VO_{2peak} ~74 ml.kg^{-1}.min^{-1}) exhibit faster oxidative recovery (~3.2 min^{-1}) when compared to sedentary individuals (VO_{2peak} ~34 ml.kg^{-1}.min^{-1}) (~1.7 min^{-1}) (Brizendine et al., 2013). In contrast, patients that share characteristics of exercise intolerance and fatigue during low-intensity work often demonstrate a reduction in muscle oxidative capacity. This has been described for physical inactivity (Ryan et al., 2013b), immobilization (Krieger et al., 1980), aging (McCully et al., 1994a; Larsen et al., 2009, 2012), chronic disease (Southern et al., 2015), and motor complete spinal cord injury (Levy et al., 1993; McCully et al., 2011; Erickson et al., 2013), as well as a variety of metabolic disorders, such as obesity (Wells et al., 2008), type 2 diabetes (Mogensen et al., 2007), and mitochondrial myopathy (van Beekvelt et al., 1999).

Trainable differences in oxidative function have been described using NIRS, where following a 4-week period of endurance training, wrist flexor muscles increased their oxidative function by 64% (Ryan et al., 2013b). Shorter bouts of training across 7–14 days also potentiate markers of mitochondrial adaptation, that underlie changes oxidative potential (Egan et al., 2013). Therefore, we have observed that repeated IPC can stimulate adaptive changes in skeletal muscle oxidative function that are replicate to changes initiated by exercise. Despite the moderate shift in oxidative capacity reported, the smaller margin for improvement in a sample population that is already healthy and trained, alongside the well-conditioned nature of the

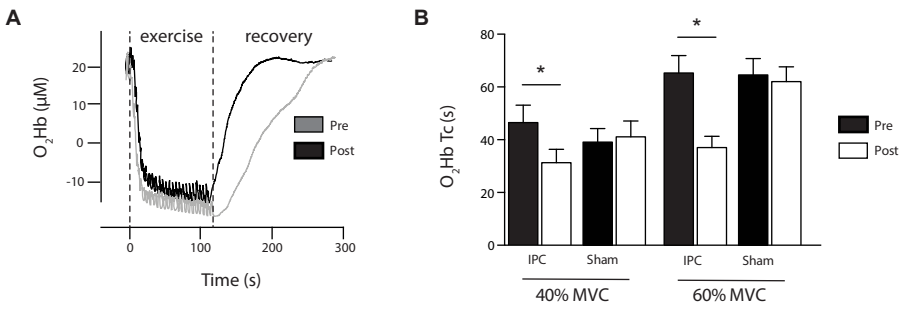

FIGURE 5 | (A) Representative exercise protocol showing submaximal intermittent plantar flexion contractions at 40% 1RM and the subsequent reoxygenation in recovery. Data shown is from NIRS O_2Hb and presented as pre (gray) and post (black) 7-day IPC. Note: recovery is faster ($P < 0.05$) following 7-day IPC. **(B)** The time constant (Tc) of recovery O_2Hb following exercise at 40 and 60% MVC is presented for IPC and Sham as pre (black) post (white). *$P > 0.05$.

TABLE 2 | Heart rate, heart rate variability, and mean arterial pressure (MAP) during rest and occlusion among IPC (n = 10) and sham (n = 10) groups.

	Sham Pre	Sham Post	IPC Pre	IPC Post
2-min resting HR ($b \cdot min^{-1}$)	62 ± 9	62 ± 9	60 ± 8	64 ± 11
2-min RMSSD (ms)	145 ± 98	96 ± 19	169 ± 133	143 ± 107
2-min SDNN (ms)	113 ± 87	62 ± 19	139 ± 136	103 ± 74
5-min occlusion HR ($b \cdot min^{-1}$)	63 ± 8	63 ± 6	61 ± 9	63 ± 9
5-min post-occlusion HR ($b \cdot min^{-1}$)	61 ± 9	62 ± 7	60 ± 9	63 ± 10
MAP (mmHg)	89 ± 10	87 ± 6	89 ± 6	88 ± 6

Values are expressed as mean ± SD. HR, heart rate; RMSSD, the square root of mean squared differences of successive normal RR intervals; SDNN, standard deviation of the normal RR intervals; MAP, mean arterial pressure.

locomotive muscle groups examined, when compared to greater changes reported for relatively untrained wrist flexor muscles (Ryan et al., 2013b), should be noted. We would speculate that the potential for improvement in muscle oxidative capacity in the lower limbs could be much greater and more easily achieved in a sedentary or reduced mobility patient population and, thus clinically significant where exercise programs are not appropriate. While there has been limited research into the effects of repeated IPC, cardiovascular adaptations have been described when IPC is applied for 7 consecutive days (Jones et al., 2014), episodically across a four (Kimura et al., 2007), and 8-week period (Jones et al., 2015), and even twice daily application for 300 days (Meng et al., 2012). Therefore, the importance of repeated IPC as a simple, non-invasive clinical alternative to exercise prescription to enhance skeletal muscle oxidative function alongside its reported cardiovascular benefits, warrants further study.

Resting Muscle Metabolism

In addition to enhanced oxidative capacity, a corresponding ∼16% decrease in resting muscle metabolism occurred following IPC. A similar observation has been made in porcine models, where skeletal muscle energy demand was reduced following preconditioning (Pang et al., 1995). We extend this to report a sustained reduction 72 h following the final bout of IPC. This may be explained by two potential mechanisms; either a reduction in metabolic rate (reduced ATP requirement) or an increased mitochondrial efficiency (increased ATP per molecule O_2). Interestingly, no change in resting $m\dot{V}O_2$ was reported following 4 weeks of endurance exercise training of the wrist flexor muscles (Ryan et al., 2013b). However, in clinical populations, a decreased resting muscle $m\dot{V}O_2$ has been reported in patients with chronic heart failure (Abozguia et al., 2008) and patients with severe peripheral arterial disease (Malagoni et al., 2010). In these cases, chronic reductions in oxygen supply to the muscle lead to adaptive changes in the periphery, which downregulate resting $m\dot{V}O_2$. Patients with mitochondrial myopathies also present with decreased resting $m\dot{V}O_2$, in this case due to diminished oxidative function, which is compensated by increased blood flow to the muscle (van Beekvelt et al., 1999). In contrast, patients with reduced mobility, without impaired blood flow (i.e., multiple sclerosis patients), have an increased resting $m\dot{V}O_2$ (Malagoni et al., 2013). These peripheral adaptations occur to facilitate oxidative function, thus sustaining physical activity in these patients. Based on these observations, we would speculate that the enhanced oxidative function we report may enable a reduction in resting muscle metabolism via an increased mitochondrial efficiency.

Increases in VEGF and NO (Kimura et al., 2007) that have been reported following repeated IPC suggest that changes in oxidative capacity and resting metabolic rate could be linked to adaptations in mitochondrial function. During a bout of arterial occlusion, local tissue hypoxia occurs downstream of the occlusion site. Hypoxia stimulates VEGF, which has been shown to activate Akt3, ultimately facilitating nuclear localization of PGC-1α, the master regulator of biogenesis (Wright et al., 2008). In this case, it could be hypoxia *per se* that elicits an enhanced mitochondrial capacity, thereby improving oxidative function, both at rest and following exercise. An alternate explanation is that repeated bouts of ischemic exercise, independent of systemic hypoxia, augment mRNA content of PGC-1α, and markers of oxidative stress (Christiansen et al., unpublished). Through generation of reactive oxygen species (ROS) and subsequent activation of AMPK pathways, mitochondrial function is augmented. However, it important to note that excessive ROS production leads to oxidative stress, which has been implicated in the pathophysiology of cardiovascular diseases. Therefore, future work will need to establish the pathway by which augmented oxidative function is supported. A future consideration is the emerging role of post-translational modifications of mitochondrial proteins involved in oxidative phosphorylation and how hypoxic and ischemic stress may modulate their function (Hofer and Wenz, 2014).

Vascular Adaptations Following IPC

We extended our primary hypothesis to speculate that if we found an improvement in oxidative function following repeated IPC, then this would translate to the exercising muscle. Acute application of IPC improves muscular endurance as evidenced by accelerated muscle deoxygenation kinetics (Kido et al., 2015; Tanaka et al., 2016). NIRS has also been used to describe the amplitude of response of HHb and tHb during exercise. The HHb signal, which is regarded as blood volume insensitive, is reported to reflect the balance between local oxygen delivery and utilization, meaning it can report muscle fractional oxygen extraction during exercise (De Blasi et al., 1993; Grassi et al., 2003). During intermittent isometric contractions, HHb was significantly reduced by ∼11–15% at differing exercise intensities following IPC. There are various interpretations of this finding such as a reduction in capillary recruitment during the exercise bout, a change in capillary-venous heme concentration, a reduction in muscle oxygen consumption, greater oxygen delivery, or a combination of these factors. That tHb remained unchanged, may suggest that oxygen delivery was increased following IPC via a vascular or vasodilatory mechanism.

The recovery of muscle oxygenation during a non-occluded state following exercise has also been used to evaluate oxidative

function (Chance et al., 1992; McCully et al., 1994b). A delay in recovery following exercise is due to both the time course required for PCr resynthesis, provided that muscle pH remains relatively stable, and the delivery of oxygen (Chance et al., 1992; McCully et al., 1994b). During recovery from exercise, the balance between oxygen supply and oxygen demand shifts in favor of oxygen supply as bioenergetic resources are restored (Chance et al., 1992). In patients with peripheral vascular disease, oxygen resaturation is lengthened in the lower limb due to an inhibition of blood flow (McCully et al., 1994a, 1997). Therefore, the faster reoxygenation kinetics noted in the IPC group following exercise at both intensities (∼32–43%), may reflect first, an enhanced oxidative capacity and, second, increased oxygen delivery via a vasodilatory mechanism or vascular adaptation. Our data supports previous findings where simultaneous increases endothelial–dependent vasodilation (∼9%) and vascular conductance (∼14%) have been described following daily exposure to IPC for 7 days (Jones et al., 2014). Indeed, similar observations have also been made following intermittent exposure over 4 weeks (Kimura et al., 2007) and 8 weeks (Jones et al., 2015). The primary mechanism that has been proposed to mediate such changes has identified the sheer stress exerted on the blood vessels following a period of ischemia and subsequent reperfusion, as a major stimulus for microvascular adaptation and endothelial function due to the subsequent increase in blood flow following reperfusion (Green et al., 2010; Tinken et al., 2010). Interestingly, Jones et al. (2014) also reported vasculature adaptations in remote areas that were not directly exposed to the repeated ischemic stimuli, suggesting that circulating factors such as VEGF or endothelial progenitor cells (Kimura et al., 2007) may elicit both local and systemic effects. Much work remains to be carried out to understand the effect of IPC on local and systemic vascular and oxidative function.

Systemic Cardiovascular Effects of IPC

Reductions in systolic and diastolic blood pressure have been observed following repeated IPC (Madias, 2011; Jones et al., 2014). However, we found no changes in blood pressure. There are inconsistencies in the literature, with several studies reporting no changes in blood pressure (Kimura et al., 2007; Madias, 2015) suggesting that more evidence is needed to understand the systemic effects of IPC. In addition, no changes in resting heart rate or autonomic function were noted between groups, as indicated by measurements of HRV. This was unanticipated, given the systemic effects of IPC on parasympathetic nervous system activity that have been demonstrated (Enko et al., 2011). Systemic cardiovascular protective effects of acute IPC are temporal in nature, with an early (1–4 h) and late (24–72 h) protective window (Yellon and Baxter, 1995). We postulate that the testing protocol utilized, whereby observations were made 72 h following the final bout of IPC, could also have permitted a partial decay in systemic cardiovascular and hemodynamic markers described. In addition, the cohorts studied were healthy, active, and already well-conditioned to exercise, which may have reduced the potential for changes in cardiovascular function. It would appear that the nature and time-course of the applied stimulus may need to be examined in more detail. It may be that systemic effects occur acutely after IPC or that repeated daily frequency is needed for these improvements to be sustained.

Risks Associated With IPC

Prolonged ischemia and reperfusion generates local tissue damage and can lead to endothelial injury, which may further impede vascular blood flow (Thijssen et al., 2016). While the implementation of IPC using a similar 7-day protocol prior to an ischemia-reperfusion injury has been shown to be effective in protecting against endothelial dysfunction (Luca et al., 2013), the question remains as to what is regarded as a safe dose. In addition, IPC has been shown to increase pro-inflammatory markers of leukocyte activation which could be of potential risk to cardiac patients (Zaugg and Lucchinetti, 2015). Therefore, the long-term safety and optimal dose of IPC remains a real consideration in clinical settings where underlying vascular issues could be compounded. Based on current evidence, when administered as a series of brief occlusions, little known side effects have been reported for a single application, nor for repeated applications up to 300 days (Meng et al., 2012). IPC also remains an effective technique to elicit cardioprotection in patients undergoing cardiac surgery.

Limitations

A potential confounding factor in this study was the control over the level of exercise fitness in the individuals tested. This may have impacted the ability to enhance skeletal muscle function using IPC. We reported on average 4 h/week exercise that consisted of running or team sport training. However, further insight into the effectiveness of IPC to enhance skeletal muscle oxidative function in athletic populations, sedentary and/or clinical populations with reduced muscle activity, is needed. For the IPC stimulus itself, the optimal strategy in terms of number of repeated applications required, length of occlusion, number of occlusions, and timings of protection to elicit these oxidative changes, as well as safety, presents future questions for the scientific community. In addition, the remote effects on skeletal muscle not directly exposed to the ischemic stimuli also warrant further study. Heterogeneity of blood flow under the NIRS optode may also have impacted measurements of skeletal muscle oxidative function. We attempted to minimize this by standardizing placement of the optode and maintaining standard laboratory conditions for all testing. NIRS signal penetration is estimated to be one-half of the interoptode distance (in this case 35 mm), therefore, we monitored participants to ensure that subcutaneous adipose tissue was $>$ 15 mm to limit absorption and scattering of light. Additional studies using multiple-source detector probes would help further validate our findings. Finally, the contribution of myoglobin to the NIRS signal remains controversial, with suggestions ranging from 20% at rest to up to 70% during exercise (Marcinek et al., 2007; Davis and Barstow, 2013). Separation of these spectra cannot be performed using the NIRS device used in this study. Therefore, future work will need to discriminate whether these contributions affect the measures of oxidative function performed in this study.

CONCLUSION

Skeletal muscle is highly adaptable to multiple physiological stimuli. Here, repeated bouts of IPC stimulus over 7 consecutive days increased skeletal muscle oxidative capacity, decreased resting muscle metabolism, and enhanced microvascular oxygenation. It is clear that the adaptations reported may have resulted from changes in hemodynamics induced by a single bout of IPC, activation of molecular pathways, or diffusible factors that mediate protective effects. Repeated activation of these mechanisms over 7 days could explain the long-term benefits beyond the reported acute late phase of protection following IPC (+ 72 h). Enhancement of skeletal muscle oxidative potential, described here, has not been previously reported following repeated IPC but may suggest a potential longer term effect on muscle performance. Future studies will need to address the mechanisms by which IPC enhances oxidative function at a cellular level, although it is likely that changes in mitochondrial function contribute to these effects. These findings will be of particular interest to athletic and clinical populations.

AUTHOR CONTRIBUTIONS

OJ, SP, and MW conceived and designed the research. OJ, SP, MW, and JP acquired, analyzed, and interpreted the data. OJ, SP, MW, and JP drafted, edited, and revised the manuscript. OJ, SP, MW, and JP approved the final version of manuscript. All authors agreed to be accountable for all aspects of the work.

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frontiers in Physiology

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Short–Term Preconditioning With Blood Flow Restricted Exercise Preserves Quadriceps Muscle Endurance in Patients After Anterior Cruciate Ligament Reconstruction

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Surgical ACL reconstruction performed with a tourniquet induces compression and ischemic stress of the quadriceps femoris (QF) muscle which can accelerate postoperative weakness. Given that low-load blood flow restricted (BFR) exercise is potent in enhancing muscle oxygenation and vascular function, we hypothesized that short-term preconditioning with low-load BFR exercise can attenuate QF muscle endurance deterioration in the postoperative period. Twenty subjects undergoing arthroscopic ACL reconstruction performed 5 exercise sessions in the last 8 days prior to surgery. They were assigned into either BFR group, performing low-load BFR knee-extension exercise, or SHAM-BFR group, replicating equal training volume with sham occlusion. Blood flow (near-infrared spectroscopy) and surface EMG of QF muscle during sustained isometric contraction at 30% of maximal voluntary isometric contraction (MVIC) torque performed to volitional failure were measured prior to the intervention and again 4 and 12 weeks after surgery. There was an overall decrease ($p = 0.033$) in MVIC torque over time, however, no significant time-group interaction was found. The time of sustained QF contraction shortened ($p = 0.002$) in SHAM-BFR group by 97 ± 85 s at week 4 and returned to preoperative values at week 12. No change in the time of sustained contraction was detected in BFR group at any time point after surgery. RMS EMG amplitude increased ($p = 0.009$) by $54 \pm 58\%$ at week 4 after surgery in BFR group only. BFm increased ($p = 0.004$) by $52 \pm 47\%$ in BFR group, and decreased ($p = 0.023$) by $32 \pm 19\%$ in SHAM-BFR group at week 4 after surgery. Multivariate regression models of postoperative changes in time of sustained QF contraction revealed its high correlation ($R^2 = 0.838$; $p < 0.001$) with changes in BFm and RMS EMG in the SHAM-BFR group, whereas no such association was found in the BFR group. In conclusion, enhanced endurance of QF muscle was triggered by combination of augmented muscle fiber recruitment and enhanced muscle perfusion. The latter alludes to a preserving effect of preconditioning with BFR exercise on density and function of QF muscle microcirculation within the first 4 weeks after ACL reconstruction.

Keywords: disuse muscle atrophy, blood flow restricted exercise, arthroscopic ACL reconstruction, ischemic preconditioning, quadriceps femoris, muscle endurance, muscle perfusion

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INTRODUCTION

Quadriceps femoris (QF) muscle weakness is the major cause of poor functional status of patients following otherwise successful anterior cruciate ligament (ACL) reconstruction (Lindstrom et al., 2013; Thomas et al., 2015). Hence, the primary goal of postoperative rehabilitation is to restore normal muscle activation and function as soon as possible. However, despite best efforts from both patient and physiotherapist during the rehabilitation process, considerable impairments of QF muscle often persist for several months after the reconstruction (Kim et al., 2010). The deficits in muscle strength and endurance contribute to altered movement patterns of the involved limb and thus increase the risk of early onset of knee osteoarthritis (Palmieri-Smith and Thomas, 2009). Rather low efficiency of standard postoperative rehabilitation has diverted the focus of clinical research in recent years toward the so-called preconditioning, or prehabilitation, exercise programs. They aim to increase lower limb muscle strength prior to surgery, which is believed to substantially attenuate the deterioration of muscle function in the aftermath. Although temporary enhancements of muscle strength and function can be clearly gained with various preconditioning programs (Keays et al., 2006; Hartigan et al., 2009), there is little evidence to support their alleged protective effect against muscle deconditioning in the postoperative period (Shaarani et al., 2013; Kim et al., 2015). The impetus for utilizing preconditioning programs comes from several studies showing strong positive associations between preoperative strength levels of knee muscles, QF in particular, and the successful long-term outcome of ACL reconstruction (Eitzen et al., 2009; Logerstedt et al., 2013). However, focusing only on maximal muscle strength gains prior to surgery may not give the best results. Our recent analysis has shown that the level of preoperative QF muscle endurance, and not its maximal strength, is the strongest predictor of muscle atrophy in the first 4 weeks after ACL reconstruction (Grapar Žargi et al., 2017). Enhancing preoperative QF muscle endurance may therefore be more important for effective protection against postoperative deconditioning in these patients.

Why some patients develop severe postoperative QF muscle weakness is not fully understood. Development of postoperative muscle atrophy and dysfunction is a multifactorial phenomenon, which is mainly caused by reduced limb loading and arthrogenic muscle inhibition driven by pain and joint swelling (Rice et al., 2014, 2015). In addition, a latent ischemic-reperfusion injury, triggered by prolonged arterial occlusion during surgery, augments QF atrophy in the early postoperative period (Appell et al., 1993; Daniel et al., 1995). It can be estimated from animal models that irreversible damage to skeletal muscle develops only after 3–6 h of complete ischemia (Blebea et al., 1987; Belkin et al., 1988). However, smaller-scale edema of the vastus lateralis muscle cells and surrounding microcirculation is initiated after only 15 min of surgery performed with a tourniquet, and progresses to severe damage and death of some myocytes after 90 min of ischemia (Appell et al., 1993). Utilizing a standard ischemic preconditioning (IPC) protocol, comprised of several 3–5 min intervals of complete blood flow restriction intermittent by equal periods of reperfusion, on a resting skeletal muscle before its exposure to prolonged ischemia can reduce gross muscle damage and increase cell survival (Sullivan et al., 2009; Murphy et al., 2010). The IPC has proven successful in attenuating muscle damage and atrophy following extremity surgery in animals (Gürke et al., 1996; Papanastasiou et al., 1999); however, no evidence of its effectiveness in human surgery is currently available in the literature. Interestingly, a frequent application (twice daily) of IPC was reported to prevent atrophy and weakness of several lower limb muscles during 14-day experimental ankle cast immobilization in otherwise healthy young males (Kubota et al., 2008).

Another muscle conditioning method, utilizing intermittent muscle blood flow restriction similar to standard IPC protocol, but in combination with low-load resistance exercise (low-load BFR exercise), has been extensively studied in recent decades. In healthy humans, the low-load BFR exercise was proven to be as effective as conventional high-load resistance exercise in increasing muscle mass and strength (Takarada et al., 2004; Abe et al., 2006). It was also demonstrated to be superior in enhancing local muscle endurance to equal exercise with normal blood flow (Takarada et al., 2002; Kacin and Strazar, 2011; Sousa et al., 2017). The endurance boosting effect of BFR exercise is closely related to enhanced muscle vascular function (Patterson and Ferguson, 2010; Hunt et al., 2013) and oxygenation (Kacin and Strazar, 2011). It was also shown to attenuate muscle atrophy during experimental limb unloading (Clark et al., 2006) and in patients after ACL reconstruction (Ohta et al., 2003), although evidence of no effect for the latter also exist (Iversen et al., 2016). Despite shorter time under occlusion compared to IPC, and the fact that complete arterial occlusion is usually not achieved prior to commencement of muscle contractions, low-load BFR exercise can nevertheless pose a short, but very intensive ischemic stimulus to the muscle tissue. Depletion of oxygen and accumulation of metabolites in the muscle are augmented by vigorous muscle activity (Suga et al., 2010), while muscle perfusion is progressively decreased due to build-up of intra-muscular pressure (Kacin et al., 2015), which can result in complete arterial occlusion in the muscle toward the end of exercise set. The intensity of ischemia-reperfusion stimulus can be modulated by various parameters of low-load BFR exercise protocol. If performed with combination of wider cuffs and higher pressure, longer time under occlusion and reperfusion periods between sets, BFR exercise induces stimulus similar to standard IPC, while, at the same time, provides substantial neuromuscular and metabolic stimuli of resistance exercise. We therefore presume that simultaneous activation of these two distinct, but partly overlapping, physiological stimuli would most effectively prevent postoperative dysfunction of QF muscle. However, our first study of short-term preconditioning of QF muscle with such low-load BFR exercise protocol has not detected a significant attenuation of atrophy, or loss of maximal strength, in the first 12 weeks following ACL reconstruction (Grapar Zargi et al., 2016). When analyzing possible reasons for no effect of the intervention, we realized that by focusing on maximal muscle strength we may have overlooked its more plausible preventative effect. Given that low-load BFR exercise is

very potent in enhancing skeletal muscle endurance and vascular function in healthy subjects, it is likely that the preconditioning with BFR exercise would have more protective effect on the same in patients exposed to prolonged surgery with a tourniquet.

We thus hypothesized in the present study that the primary effect of preconditioning with low-load BFR exercise would be to preserve QF muscle endurance and microvascular function during the first 12 weeks after ACL reconstruction and that the changes in endurance are strongly associated with changes in muscle blood flow and pattern of muscle activation.

MATERIALS AND METHODS

Ethical Approval

The study protocol was approved by the Republic of Slovenia National Medical Ethics Committee (permit No. 62/05/12, date of approval: 05/30/2012) and carried out in accordance with the Declaration of Helsinki. All patients included signed a written consent of voluntary participation after receiving detailed written and oral information on the study. Patient recruitment and surgical procedures took place at Orthopedic Department of University Medical Centre Ljubljana, whereas all performance tests and exercise training interventions were conducted at the university-based Laboratory of Physiotherapy Research.

Study Population

This study is a prospective, single-center, quasi-randomized trial controlled by SHAM-BFR intervention. Volunteers were selected and recruited among patients scheduled for an arthroscopic ACL reconstruction with ipsilateral hamstrings autographs ACL reconstruction. In total, 83 patients were assessed for eligibility to enter the study. Initially 24 patients were recruited for the study and 20 patients (16 males and 4 females) fully completed the protocol. Four patients were excluded from the final analysis due to additional surgical procedures requiring modification of post-operative rehabilitation program. The inclusion criteria were: age from 18 to 45 years, ACL tear of more than 6 months with sufficient range of motion to perform exercise (active extension deficit $<5^{\circ}$, active flexion $\geq 120^{\circ}$), pain level during exercise ≤ 20 on Visual Analogue Scale (0–100 mm) and no previous surgeries to the affected knee. The exclusion criteria were any concomitant intra-articular pathology that would require open surgery or prolonged postoperative unloading, a significant damage to the articular cartilage, additional neuromuscular impairments, spine or other lower limb injuries, presence or history of any vascular diseases or deep vein thrombosis.

Surgical Intervention and Rehabilitation

All patients underwent surgery at the same institution under spinal anesthesia by two experienced orthopedic surgeons. During surgery, a pneumatic tourniquet inflated to minimum of 300 mmHg was used on the proximal part of the thigh to completely occlude the blood flow. Surgery was performed on five dominant and five non-dominant legs in each group, where the limb dominance was determined according to the patients' self-reported preference for kicking and take-off activities. An arthroscopic single bundle ACL reconstruction was performed using a double stranded ipsilateral semitendinosus-gracilis autograft. In 12 out of 20 patients, additional partial meniscectomy was performed during surgery. Postoperative rehabilitation began on the first postoperative day, when the patients were allowed to ambulate with full weight-bearing and received functional neuromuscular electrical stimulation of thigh muscles combined with volitional isometric contractions. No postoperative braces or crutches were used. During the first 5 weeks, all patients followed identical postoperative protocol (3 times per week, cryotherapy, passive and active ROM exercises, manual joint mobilization, open and closed kinetic chain resistance exercises, balance and proprioceptive exercises) supervised by appointed physiotherapist at the designated outpatient rehabilitation unit, followed by a 14-day intensified rehabilitation program (daily sessions of individual and group therapeutic exercise for gaining full knee ROM, muscle strength and joint stability) in a spa resort. The appointed physiotherapists in both facilities were blinded of the patients' group.

Intervention

The short-term preconditioning intervention with low-load BFR exercise was designed to (i) deliver an IPC-induced protective effect on subsequent ischaemic-induced injury and (ii) increase muscle strength and endurance, as reviewed in the introduction. To induce ischemia-reperfusion stimulus similar to standard IPC, the BFR exercise protocol utilized in our patients comprised a combination of wider cuffs and moderate pressure, longer time under occlusion and longer reperfusion periods between sets. To emphasize the preventative effects of preconditioning, the last training session was performed within 48 h prior to surgery. To overcome patients' low tolerance for mechanical joint loading and assure the minimal required exercise volume and intensity of each session (Wernbom et al., 2009), multiple sets of exercise with very low resistance and high number of repetitions performed to volitional failure was used. Patients were asked not to change their routine regarding regular daily physical activities during the intervention period. The same BFR exercise protocol is described in detail in previous study from our lab (Grapar Zargi et al., 2016).

Patients conducted 5 exercise sessions, equally distributed during the last 8 days before the surgery. One patient group performed blood flow restricted exercise (BFR group) and the other group performed the exercise with sham blood flow restriction (SHAM-BFR group). A counterbalanced quasi-randomization of the patients was performed to match patients between groups by sex, overall score of Lysholm Knee Scoring Scale for patient's self-assessment of knee function (Tegner and Lysholm, 1985) and body mass index; thus, the first 2 patients were assigned to BFR group and the next two to SHAM-BFR group. This proceeding was repeated until each group comprised 10 patients who completed the entire experimental protocol. This protocol ensured equal volume of mechanical work performed by the two groups.

The patients performed unilateral resisted knee extension in an open kinetic chain on a leg-extension machine (Sokol Gym, Slovenia, EU) with ACL deficient leg only. The non-injured leg was not trained and served as a control for calculation of

strength deficits. Patients in BFR group performed 6 sets of knee-extension exercise to volitional failure at each exercise session. A metronome was set to 56 bpm to determine the rhythm and speed; one beat for eccentric and one beat for concentric phase of muscle contraction. The 40-repetition maximum was set individually for each patient before the first exercise session by trial and error. Only after the initial warm-up with 10–15 repetitions with 0.5 kg load, were the tourniquets inflated to 150 mmHg and after 30 s of resting occlusion the patient started to exercise. After the first, the third and the fifth set, 45 s of rest without reperfusion was allowed. Only after the second and the fourth set, the tourniquets were deflated to allow 90 s of reperfusion. The patients in SHAM-BFR group followed the same exercise protocol and replicated the number of repetitions per set achieved by his/her pair from the BFR group.

Muscle blood flow was restricted with a 14-cm wide contoured pneumatic tourniquet cuff (VariFit Conture Thigh Cuff, Delfi Medical Innovations, Canada) placed around the proximal part of the thigh and connected to a pressure regulating system (Portable Tourniquet System, Delfi Medical Innovations, Canada). A uniform cuff pressure of 150 mmHg used in the BFR group was set based on results of series of prior pilot experiments performed in our lab on healthy subjects. This pressure level allows for maximal QF muscle deoxygenation at the end of each exercise set (measured with NIRS), without compromising normal muscle activation due to pain and discomfort elicited at the site of tissue compression (authors' unpublished data). In SHAM-BFR group, the occlusion pressure was set to only 20 mmHg, therefore not restricting muscle blood flow.

Outcome Measures

Assessment of muscle strength and endurance comprised measurements of maximal volitional isometric contraction (MVIC) torque and time of sustained submaximal isometric contraction, surface EMG of vastus medialis muscle and oxygenation and hemoglobin kinetics of vastus lateralis muscle. The measurements were performed prior to the preconditioning protocol (PRE) and repeated at week 4 (POST wk4), with exclusion of MVIC torque, and at week 12 (POST wk12) after surgery.

Muscle Maximal Isometric Torque

The patients performed MVIC torque measurement on static dynamometer (Isometric Knee Dynamometer, S2P d.o.o., Ljubljana, Slovenia) in a sitting position with 60° of knee flexion and stabilized over pelvis with security belt. Loading of ACL has been shown to be minimal in this joint position (Escamilla et al., 2012), which allowed safe testing also postoperatively. Three attempts of 3–4 s MVIC were performed with 3 min rest period in-between to avoid muscle fatigue. Strong verbal encouragement was systematically given to motivate subjects. The highest torque of the three attempts averaged over 1 s was used for further analyses.

Muscle Isometric Endurance

The QF endurance test was performed in the same position as described above. The 30% of preoperative MVIC torque was calculated for each individual and set as a target for evaluating endurance. Based on our pilot experiments, this level of sustained contraction proved to be demanding enough and feasible in both preoperative and postoperative periods, which allowed comparison of the contraction times at equal absolute load at all three time points. A line marking the target of 30% of individual's MVIC torque was provided on a computer screen set in front of the patient for visual feedback. The patient followed the target line as closely as possible during the test. Another line marking the lowest limit for test termination at 90% of the target value was also shown on the screen. The test was terminated at patient's volitional failure, or if muscle force dropped below the limit for more than 3 s despite strong verbal encouragement. The time of sustained contraction at target was used as measure of the endurance.

Surface Electromyography

The activation of vastus medialis muscle was measured by surface EMG during the endurance test. Electrodes were positioned according to SENIAM standards (Hermens et al., 2000) to avoid overlap of the innervation zones and cross-talk of the muscles. Skin was shaved, lightly abraded and cleaned with alcohol to reduce electrical impedance <5,000 Ohms. A pair of bipolar surface electrodes Ag-AgCl (Red Dot 2560, 3M, USA) was placed longitudinally on the muscle belly. EMG activity was amplified with four-channel tethered remote monitoring unit at a sampling rate of 1,000 Hz, input impedance 2 MΩ, bandwidth of 1–500 Hz (TEL 100C and MP150WS, Biopac Systems, USA). The raw EMG data were filtered with a high pass infinite impulse response digital filter at a cutoff frequency of 20 Hz (Acqknowledge 3.9.2., Biopac Systems Inc.). Root mean square (RMS) smoothening of the filtered signal with 3,000 ms time window was used to quantify EMG amplitude. The frequency and power analysis included fast Fourier transformation of the filtered EMG signal, followed by median frequency calculation (F_{med}) for the entire contraction time. Standard normalization of EMG amplitude to MVIC values was neither feasible nor appropriate due to potential injury of reconstructed ACL graft at POST WK4 during maximal QF contraction and substantial decrease in postoperative MVIC torque. EMG amplitude at the same absolute torque, corresponding to 30% of the initial MVIC torque, was compared at all three time points.

Muscle Blood Flow

Muscle blood flow (BF_m) in vastus lateralis muscle was measured with near-infrared spectroscopy (NIRS) during the endurance test (Oxymon Mk III, Artinis Medical Systems, Arnheim, The Netherlands), based on absorption of infrared light in the muscle tissue (Ferrari et al., 2004). Concentrations of oxygenated ($[O_2Hb]$) hemoglobin and deoxygenated hemoglobin ([HHb]) in the muscle were measured with 760 and 850 nm wavelengths, respectively. The skin on the muscle belly was shaved, lightly abraded and cleaned with alcohol before plastic holder for two optodes was fixed to the skin with double-sided adhesive tape. The interoptode distance was set between 35 and 50 mm, depending on the subject's skinfold thickness at the site of optode placement. To minimize the influence of daily variations, the

values are reported as deltas (Δ). Resting values were acquired during 10 min of quite rest in the same sitting position as used for testing. To assess muscle blood flow a 30-s venous occlusion (cuff pressure = 60 mmHg) was repeated twice with 15 s pause in-between. The data were averaged over the two measures. To obtain the resting values the procedure was performed from 4 to 6 min of the rest period. To obtain post-exercise values the procedure was performed immediately after the endurance test, starting within 5 s after cessation of sustained isometric contraction. Signals were acquired at a sampling rate of 10 Hz and recorded. The raw signal was filtered with 2-sec moving average (Oxysoft 2.0.47 software, Artinis Medical Systems, Arnheim, The Netherlands) to remove movement artifacts. Blood flow was calculated using the following formula (Van Beekvelt et al., 2001):

$$BF_m = (((\Delta[tHb] \cdot 60)/(([Hb] \cdot 1000/4)) \cdot 1000)/10$$

where [tHb] is expressed in $\mu M \cdot s^{-1}$ and converted to milliliters of blood per minute per 100 milliliters of tissue ($mL \cdot min^{-1} \cdot 100mL^{-1}$) and group reference values for hemoglobin concentration ([Hb] in $mmol \cdot L^{-1}$) of males and females obtained from the literature are used. The molecular weight of Hb (64.458 $g \cdot mol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) was also taken into account. The intra-observer coefficient of variation was 8.2%.

Statistical Analysis

All statistical analyses were performed with Statistica software (Version 12, StatSoft Inc., Tulsa, Oklahoma, USA). A threshold of significance was set at $\alpha < 0.05$ for all tests. Unless stated otherwise, variables are expressed as means ± standard deviation (SD).

Sample Size Estimation

Estimation of minimal sample size at statistical power level of ≥0.80 ($\beta \leq 20\%$) was calculated for two-way ANOVA test based on standardized effective size for the main observed outcome measure of QF muscle endurance, i.e., the time of sustained isometric contraction. A predicted preoperative mean of 190 s with standard deviation of 30 s, determined by our pilot experiments, was used for calculations. By assuming an interaction of 30% difference between the groups over time, the estimated minimal number of subjects was nine.

Comparison of Means

Normality of distribution of each data set was analyzed with Shapiro-Wilk test and parametric statistical analysis was deemed appropriate. The initial patients' characteristics of the groups were tested with the Student's *t*-test. Comparison of means in contraction time, MVIC torque, RMS EMG, F_{med} and BF_m was made with two-way (group × time) ANOVA with repeated measures on the time factor. In case of significant main effect, the *post hoc* pairwise comparisons were made with the Tukey's honestly significant difference test.

Regression Analyses

The regression analysis aimed to elucidate the extent of association between changes in QF endurance and changes in muscle activation and perfusion. A multivariable linear regression analysis of changes (Δ) in time of sustained contraction as the dependent variable was performed first from the pooled data of both groups and then separately for each group. The Δ RMS EMG and Δ BF_m were calculated for periods PRE to POST WK4 and POST WK4 to POST WK12. Both parameters were simultaneously introduced to the models as independent variables. The coefficient of determination (R^2), model's intercept, regression coefficient (b), standardized regression coefficient (β) and *p*-value were computed and reported. To assess independent association between dependent and each independent variable, the univariate linear regressions were performed and Pearson's correlation coefficients (r) calculated.

RESULTS

There were no significant differences in body mass index (BFR = 24.3 ± 3.9 $kg \cdot m^{-2}$; SHAM-BFR = 23.9 ± 2.9 $kg \cdot m^{-2}$), age (BFR = 34 ± 6 years; SHAM-BFR = 35 ± 5 years) and Lysholm composite score (BFR = 78 ± 11; SHAM-BFR = 76 ± 16) between the groups. The average duration of the surgery was 71 min (range 52–113 min) with no significant difference between the groups (BFR = 69 ± 9 min; SHAM-BFR = 71 ± 14 min).

Muscle Isometric Strength and Endurance

Absolute values of QF strength and endurance outcome measures and results of statistical analysis are presented in **Table 1**. There was an overall decrease in absolute MVIC torque over time, however no significant time-group interaction was found. Likewise, there was an overall change in MVIC torque deficit of OP leg in time, with non-significant time-group interaction. Twelve weeks after surgery, the deficit increased to −19 ± 14% in BFR group and −20 ± 12% in SHAM-BFR group, with no difference between the groups (**Figure 1A**).

The time of sustained contraction showed significant interaction of time and group factors (**Table 1**). It was significantly shorter ($p < 0.001$) in SHAM-BFR group at POST WK4 compared to the baseline value, whereas in BFR group it did not decrease significantly, resulting in a significantly different ($p = 0.029$) change in the parameter between the groups at POST WK4. At POST WK12, the time of sustained contraction remained at similar level in BFR group and returned to preoperative values in SHAM-BFR group (**Figure 1B**).

Surface EMG and Muscle Blood Flow

Absolute values and results of statistical analysis of surface EMG and muscle blood flow are presented in **Table 1**. The amplitude of RMS EMG showed significant ($p = 0.001$) interaction of time and group factors. It significantly ($p = 0.009$) increased in BFR group by 54 ± 58% at POST WK4 and returned to preoperative values at POST WK12. No significant difference in RMS EMG was noted in SHAM-BFR at any postoperative time point. There was a tendency ($p = 0.057$) for significant difference in the amplitude of RMS EMG between groups at POST WK4 (**Figure 2A**).

TABLE 1 | Mean (SD) values of quadriceps femoris muscle strength, endurance, EMG activity and blood flow in BFR and SHAM-BFR group during the 12-week postoperative period.

	BFR group ($n = 10$)			SHAM-BFR group ($n = 10$)			*p*-value		
	PREOP	POST WK4	POST WK12	PREOP	POST WK4	POST WK12	Time	Group	Interaction
MUSCLE STRENGTH AND ENDURANCE									
MVIC torque (Nm)	238 ± 83	/	196 ± 48	210 ± 44	/	181 ± 34	0.002	0.350	0.519
Time of contraction (s)	145 ± 47	153 ± 54	199 ± 71	192 ± 93	95 ± 90	187 ± 94	<0.001	0.902	0.014
EMG ACTIVITY AND MUSCLE PERFUSION									
RMS EMG (mV)	0.274 ± 0.163	0.391 ± 0.293	0.256 ± 0.160	0.224 ± 0.096	0.187 ± 0.103	0.210 ± 0.086	0.049	0.125	0.001
F_{med} (s)	53.2 ± 10.9	45.7 ± 10.1	45.9 ± 4.4	49.9 ± 5.9	41.7 ± 5.8	44.1 ± 3.7	<0.001	0.286	0.730
BF_m (mL. min^{-1}. $100mL^{-1}$)	1.26 ± 0.34	1.92 ± 0.81	1.49 ± 0.75	1.53 ± 0.44	0.97 ± 0.20	1.36 ± 0.32	0.907	0.176	<0.001

MVIC, maximal volitional isometric contraction; RMS EMG, root mean square of electromyograhic amplitude; F_{med}, median frequency of EMG spectrum; BF_m, muscle blood flow; POST WK4, 4 weeks after surgery; POST WK12, 12 weeks after surgery; BFR, blood flow restriction; SHAM-BFR, sham blood flow restriction.

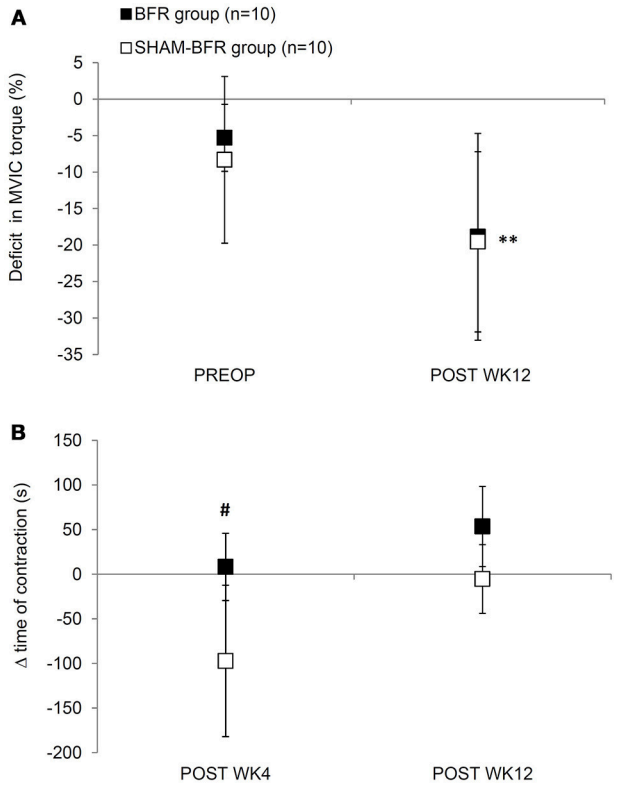

FIGURE 1 | Mean (SD) deficits in MVIC torque **(A)** and changes (Δ) in time of sustained contraction at 30% MVIC torque **(B)** of quadriceps femoris muscle of the affected leg in BFR and SHAM-BFR group prior to (PREOP), at 4 weeks (POST WK4) and at 12 weeks (POST WK12) after surgery. # denotes statistical difference between groups at $p < 0.05$. **denotes statistical difference compared to PREOP values at $p < 0.01$.

The F_{med} showed no significant ($p = 0.730$) interaction of time and group factors. F_{med} decreased by 14 ± 6% in BFR group ($p = 0.013$) and by 16 ± 12% in SHAM-BFR group ($p = 0.005$) at POST WK4 and tended to remain lowered at POST WK12 (BFR = 12 ± 13 %, $p = 0.016$; SHAM-BFR = 11 ± 10%, $p = 0.086$). There were no significant differences between the groups at any timepoint (**Figure 2B**).

The BF_m showed significant ($p < 0.001$) interaction of time and group factors. BF_m significantly increased by 52 ± 47% in BFR group ($p = 0.004$), and decreased by 32 ± 19% in SHAM-BFR group ($p = 0.023$), at POST WK4. There was a significant difference ($p = 0.004$) in the BF_m between groups at POST WK4 (**Figure 2C**).

Models of Postoperative Change in Muscle Endurance

A multiple regression model of pooled group data of Δ time of contraction of QF in the postoperative period revealed a significant ($p = 0.006$) relationship ($R^2 = 0.245$) with the following Equation [1]:

$$\Delta\text{Time of contraction} = (-25.453 \cdot \Delta\ \text{RMS EMG}) + (65.500 \cdot \Delta\ BF_m) + 10.718 \quad (1)$$

As shown in **Table 2**, the Δ RMS EMG had small and non-significant ($p = 0.845$) negative weight, while Δ BF_m had large and significant ($p = 0.014$) positive weight. The multiple regression model of Δ time of contraction of QF for the BFR group revealed a non-significant ($p = 0.826$) and weak relationship ($R^2 = 0.002$) between the variables (**Table 2**). In contrast, the multiple regression model of Δ time of contraction of QF for the SHAM-BFR group revealed a significant ($p < 0.001$) and strong relationship ($R^2 = 0.838$) with the following Equation [2]:

$$\Delta\text{Time of contraction} = (448.536 \cdot \Delta\ \text{RMS EMG}) + (130.149 \cdot \Delta\ BF_m) + 10.994 \quad (2)$$

As shown in **Table 2**, the both Δ RMS EMG ($p = 0.002$) and Δ BF_m ($p < 0.001$) had large and significant positive weight.

Univariate Regression Analysis

The univariate correlation of the pooled group data between of Δ time of contraction and Δ RMS EMG was low ($r = -0.330$;

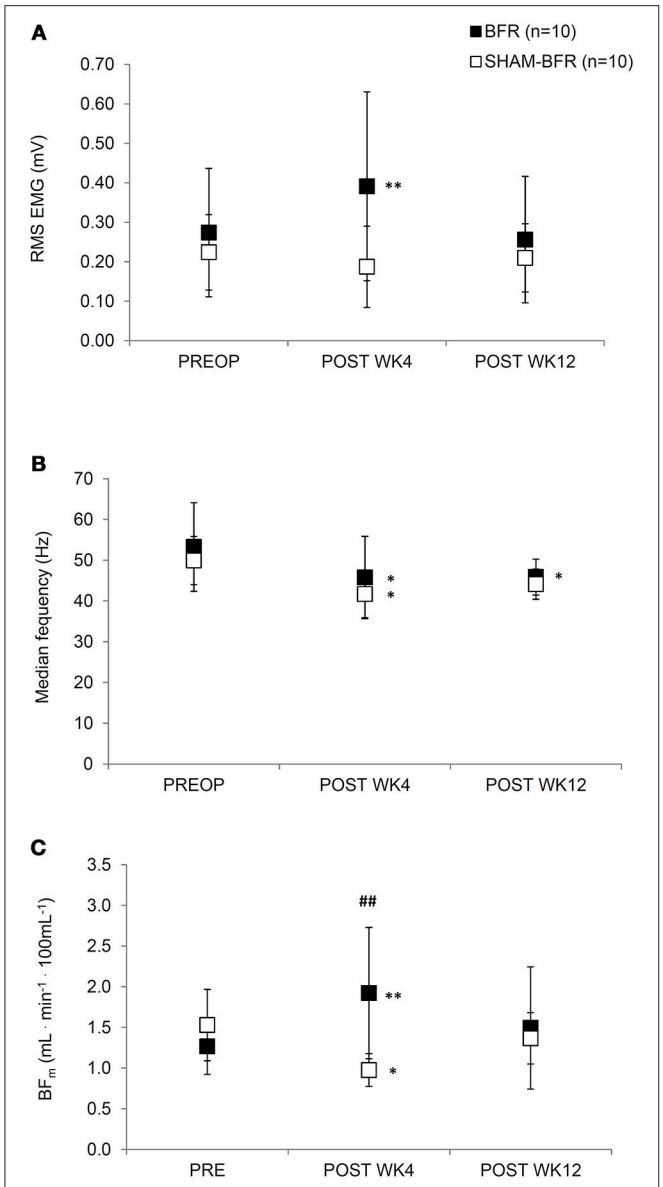

FIGURE 2 | Mean (SD) values of root mean square EMG amplitude **(A)**, median EMG frequency **(B)** and blood flow **(C)** of the affected leg during sustained contraction of quadriceps femoris muscle at 30% MVIC torque for both BFR and SHAM-BFR group prior to (PREOP), at 4 weeks (POST WK4) and at 12 weeks (POST WK12) after surgery. ## denotes statistical difference between groups at $p < 0.01$. *,**denote statistical difference compared to PREOP values at $p < 0.05$ and $p < 0.01$, respectively.

$r^2 = 0.109$) and significant ($p = 0.038$). The correlation with Δ BF_m was moderate ($r = 0.494$; $r^2 = 0.244$) and significant ($p = 0.001$) (**Figures 3A,B**). For BFR group, the correlation between Δ time of contraction and Δ RMS EMG was low ($r = -0.099$; $r^2 = 0.010$) and non-significant ($p = 0.679$); also, the correlation with Δ BF_m was low ($r = -0.023$; $r^2 = 0.001$) and non-significant ($p = 0.922$) (**Figures 3C,D**). For SHAM-BFR group, the correlation between Δ time of contraction and Δ RMS EMG was high ($r = 0.701$; $r^2 = 0.491$) and significant ($p < 0.001$); likewise, the correlation with Δ BF_m was high ($r = 0.843$; $r^2 = 0.711$) and significant ($p < 0.001$) (**Figures 3E,F**).

TABLE 2 | Multivariate linear regression models of change in quadriceps femoris (QF) muscle time of submaximal isometric contraction after ACL reconstruction for pooled group data and separately for BFR and SHAM-BFR group.

	β ± SE	b ± SE	*p*-value
POOLED GROUP DATA			
Intercept		10.718 ± 13.012	0.415
Δ RMS EMG (mV)	−0.040 ± 0.202	−25.453 ± 129.510	0.845
Δ BF_m (mL. min^{-1}. 100 mL^{-1})	0.522 ± 0.202	65.501 ± 25.378	0.014
BFR GROUP (*n* = 10)			
Intercept		25.065 ± 9.534	0.018
Δ RMS EMG (mV)	−0.276 ± 0.449	−61.409 ± 99.882	0.547
Δ BF_m (mL. min^{-1}. 100 mL^{-1})	0.210 ± 0.449	9.791 ± 20.939	0.646
SHAM-BFR GROUP (*n* = 10)			
Intercept		10.994 ± 11.882	0.368
Δ RMS EMG (mV)	0.400 ± 0.110	448.536 ± 122.931	0.002
Δ BF_m (mL. min^{-1}. 100 mL^{-1})	0.661 ± 0.110	130.149 ± 21.568	<0.001

Δ RMS EMG, change in root mean square EMG amplitude; Δ BF_m, change in muscle blood flow; BFR, blood flow restriction; SHAM-BFR, sham blood flow restriction; SE, standard error.

DISCUSSION

This is the first evidence of preventative effect of low-load BFR exercise on skeletal muscle endurance following ACL reconstruction. The results showed that the patients treated with low-load BFR exercise protocol did not display deterioration in QF muscle endurance in the first 4 weeks after surgery, whereas patients who had performed the same exercise protocol with sham occlusion demonstrated ∼50% reduction of muscle endurance. The difference in QF endurance between groups was no longer evident 12 weeks after surgery, thus the main hypothesis is only partially confirmed. As shown by multivariate regression models of changes in time of sustained isometric contraction, the decrease in QF muscle endurance was moderately correlated with decreases in BF_m (**Figures 3A,B**); the latter was also the most influential and significant individual predictor of change in QF endurance during the 12-week postoperative period. The preconditioning had neither significant nor a clinically important positive effect on QF maximal strength at any time point after surgery, which corroborates the results of our first study with a similar protocol of intervention (Grapar Zargi et al., 2016). From the parameters measured in the present study, we cannot identify explicit physiological mechanisms for these findings, however some plausible explanations can be given.

Muscle Blood Flow and Microvascular Function

A ∼50% increase in post-exercise muscle blood flow in BFR group and a ∼30% decrease in the SHAM-BFR group at 4 weeks after the reconstruction allude to changes in either vasomotor

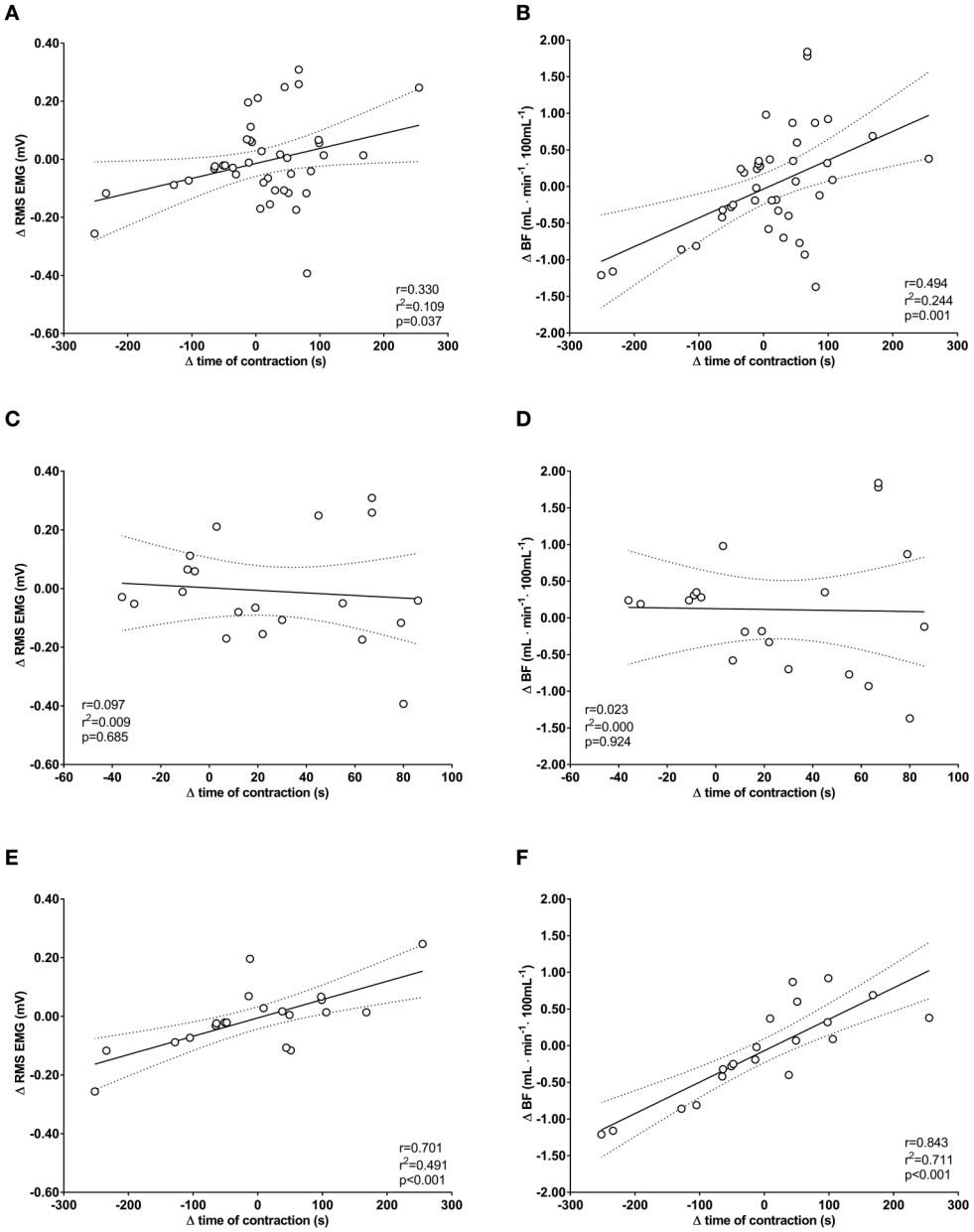

FIGURE 3 | Univariate correlations between changes (Δ) in time of sustained isometric contraction at 30% MVIC torque and changes in EMG amplitude (Δ RMS EMG) and perfusion (Δ BF_m) of quadriceps femoris muscle in the postoperative period. Plots are for pooled group data **(A,B)** and separately for BFR group **(C,D)** and SHAM-BFR group **(E,F)**.

tone regulation or density of muscle capillary network. It appears that preconditioning with BFR exercise enhanced muscle microvascular function and preserved it up to 4 weeks after surgery, whereas deterioration of microvascular function was present in patients who were preconditioned with sham intervention. Degradation of skeletal muscle capillary network follows prolonged ischemia (Hammersen et al., 1979) and delays its recovery (Appell et al., 1993), therefore deterioration of muscle blood-flow response to exercise observed in the SHAM-BFR group could be a sign of the ischemia-reperfusion damage to the QF muscle microcirculation induced by the surgical tourniquet. The changes in [tHb] acquired by near-infrared spectroscopy, on which the measurement of BF_m in our study is based on, have been previously shown to be negatively correlated ($r^2 = 0.58$, $p = 0.003$) with muscle protein oxidation after release of surgical tourniquet and can be thus regarded

as a valid predictor of ischemia-reperfusion muscle injury (Shadgan et al., 2012).

To our knowledge, no other reports of the long-term effects of preconditioning with low-load BFR exercise on atrophied muscle microvascular function or hemodynamics exists in the literature. In healthy subjects, Patterson and Ferguson (2010) reported a similar augmentation (38–47%) of muscle blood flow following a 4-week low-load BFR training of plantar flexors, despite utilizing a different method of blood flow assessment (i.e., post-occlusive hyperemia). The present results are also in line with our previous observations of improved QF muscle oxygenation following a 4-week low-load BFR exercise program, where a 120% increase in Δ[tHb], an index of gross muscle perfusion, and concomitant 56% increase in $\Delta[O_2Hb]$ was noted (Kacin and Strazar, 2011). More than 2-fold higher magnitude of increase can be attributed to a much longer training period and the fact that blood flow was reevaluated immediately after the intervention. Interestingly, very similar effects on muscle blood flow have been also reported with the use of standard IPC in healthy subjects. Different doses of IPC, ranging from very frequent application of 6 cycles of 5-min arterial occlusion and 5-min reperfusion daily for 4 weeks (Kimura et al., 2007) to less frequent application of 4 cycles of 5-min arterial occlusion and 5-min reperfusion 3 times weekly for 8 weeks (Jones et al., 2015) have been proven effective for improving forearm blood flow and skin microcirculatory function. Shorter interventions of 4 cycles of 5-min arterial occlusion and 5-min reperfusion daily for 7 days, have also been shown to have a similar protective effect (Jones et al., 2014). A recent study by Jeffries et al. (2018) demonstrated that the same 7-day IPC protocol increases oxidative function of plantar flexor muscles by ∼13% and induces faster muscle reoxygenation (∼32–43% increase in time constant of $[O_2Hb]$) following repeated isometric exercise of comparable submaximal intensity (40–60% MVIC). The latter were attributed to either an enhanced oxidative capacity or increased oxygen delivery via a vasodilatory mechanism or vascular adaptation (Jeffries et al., 2018). However, the exact mechanisms of enhanced microcirculatory function of skeletal muscle by IPC have not been fully identified as yet. It was shown on a rat model of mesentery microcirculation that reduction of leukocyte-endothelial interaction in postcapillary venules plays an important role in this regard (Erling et al., 2010). In humans, repetition of IPC stimulus was also shown to augment myocardial blood flow via endothelium-dependent vasodilation through increases in nitric oxide production (Kimura et al., 2007).

The similarity in effects on muscle endurance and microvascular function observed by standard IPC and our present results confirms the initial presumption that the IPC protocol and low-load BFR exercise impose a comparable stimulus to the skeletal muscle, at least regarding muscle microvascular function and adaptation. However, not every protocol of BFR exercise may be equally effective. Proper combination of cuff width and pressure, occlusion time, duration of reperfusion between sets and the timing of its application is likely the key to eliciting protective effects by BFR exercise. The protocol utilized in the present study comprised 6 sets of low-load exercise under occlusion at 150 mmHg with 90 s reperfusion in-between, which gives a total of 3 cycles of ∼180 s under occlusion delivered 5 times in 8 days, indicating that a significant effect on muscle microcirculatory function was achieved by substantially shorter overall ischemia-reperfusion exposure than during 7-day IPC protocols used by other researchers (Jones et al., 2014; Jeffries et al., 2018). The exact reason for the enhanced efficiency cannot be elucidated, but we presume that much higher metabolic rate and metabolite accumulation in the muscle due to its activity potentiate the ischemic stimulus.

By performing the last BFR exercise session within 24–48 h prior to surgery, we utilized the same time frame as for the late phase of protection with IPC (Loukogeorgakis et al., 2005). The attenuated decrease in BF_m strongly suggest that QF muscle microcirculatory function was successfully protected in patients preconditioned with BFR exercise. This presumption is further corroborated with the results of the multivariate regression model showing that deterioration of QF muscle endurance in SHAM-BFR group was strongly correlated with the reduction in BF_m (**Figures 3E,F**), whereas no significant correlation was found in the BFR group (**Figures 3C,D**). The reason for the difference in pattern of association of variables between the groups is unknown. It is possible that a large difference in magnitude of change in dependent variable between the groups has influenced the sensitivity of the model. It must also be considered that the general robustness of the model may not have been sufficient for detecting actual association between the variables.

Muscle Activation

No reports on long-term effects of BFR exercise on median frequency in either healthy or injured subjects can be found in the literature. There are, however, several reports on acute frequency modulation induced by different protocols of BFR that may be relevant for interpretation of our results. Previous studies, investigating the interaction between BFR exercise and patterns of neuromuscular activation, have shown that acute BFR exercise augments neural activation and recruitment of type II motor units (Moritani et al., 1992; Takarada et al., 2000; Moore et al., 2004). Pierce et al. (2006), and more recently also Fatela et al. (2016), demonstrated that higher levels of BFR elicited greater decrements in median frequency of rectus femoris, vastus lateralis, and vastus medialis muscle in a short period after exercise, which implies that the magnitude of neuromuscular fatigue is substantially larger following this form of exercise. A progressive increase in type II motor unit recruitment and reduction in fatigue would therefore be expected to be the primary long-term adaptation of BFR training. However, the uniform decrease in F_{med} of vastus medialis muscle during isometric endurance test does not suggest any differences in recruitment of type I and II motor units, or reduction in neuromuscular fatigue, between our patient groups at any time point after surgery. It must be emphasized that the F_{med} was decreased although the same absolute torque was sustained during the test. Given that QF muscle MVIC torque was reduced by ∼20% in both groups at both POST WK4 and WK12, the relative intensity of muscle contraction during the postoperative endurance tests was correspondingly increased.

Median frequency normally increases proportionally to the increase in relative intensity of muscle contraction (Croce et al., 2014), but the opposite was observed in our patient cohort. Similar paradox of decreased median frequency of the QF muscle in patients following ACL reconstruction was also observed in other studies (McHugh et al., 2001; Drechsler et al., 2006), which implies a pathological pattern of motor unit recruitment following surgery. The altered neuromuscular activation is most likely driven by altered sensory input from the damaged joint and soft tissue, which contributes to the phenomenon of arthrogenic muscle inhibition (Hurley et al., 1994).

Despite the uniform decrease in F_{med} after the surgery, BFR group presented significant increase in the amplitude of RMS EMG at POST WK4. This implies on the preserved ability to increase volitional activation of larger number of motor units to compensate for the decrease in force capacity, which in turn resulted in significantly longer times of sustained contraction compared to SHAM-BFR group. The results are in line with consensus in the literature that BFR exercise implicates greater muscular activation to maintain the same work output (Moritani et al., 1992; Takarada et al., 2000; Wernbom et al., 2009; Yasuda et al., 2009; Cook et al., 2013; Fahs et al., 2015; Loenneke et al., 2015; Fatela et al., 2016). Hypothetically, these increments in EMG amplitude may reflect an enhanced recruitment of type II motor units (Moritani et al., 1992; Takarada et al., 2000; Moore et al., 2004) due to the local accumulation of metabolites (Loenneke et al., 2015) or increase in eccentric muscle activity (Wernbom et al., 2009) during exercise with BFR. In face of no difference in F_{med} between the groups, an increased portion of active type II motor units in BFR group following surgery cannot be clearly confirmed or refuted. This paradox needs to be scrutinized by future experiments.

Potential Changes in Central Motor Drive

The increase in QF muscle endurance and RMS EMG amplitude observed in the BFR group may be also explained by modulations in central motor drive. Given that neither subjective evaluation of perceived body exertion nor any direct measure of central activation ratio were used in the present study, the potential mechanisms discussed below must be regarded as speculative.

Similar 3–8% improvements in endurance performance have been reported immediately after application of standard IPC protocol in cats (Phillips et al., 1997) and healthy humans (Chrisafulli et al., 2011; Cruz et al., 2015; Patterson et al., 2015; Griffin et al., 2018). Several authors hypothesized that increased muscle endurance observed after IPC may be attributed to enhanced ability to utilize central reserve, which represents unused potential of muscle activation from higher motor centers (Chrisafulli et al., 2011; Cruz et al., 2015; Patterson et al., 2017). The IPC supposedly decreases sensitivity of type III and IV polymodal receptors in the muscle, which enables additional activation of central reserve and subsequently preserves muscle force production (Cruz et al., 2017). Given that BFR exercise, especially if performed to volitional failure, induces greater volitional effort and muscle discomfort than a free-flow exercise, it may enhance utilization of central reserve and decrease central fatigue by similar mechanisms as hypothesized for IPC. Given that the central fatigue has been accounted for up to 65% of the decrease in force production during sustained muscle contraction of similar intensity as used in our endurance test (Smith et al., 2007), a decrease in central fatigue may have played an important role in enhanced isometric QF endurance of the patients preconditioned with BFR exercise.

Limitations and Prospects of Future Research

The following limitations need to be considered when interpreting the reported findings. Firstly, there are several equally important initial patients' characteristics (level of physical activity, leg-dominance, preoperative muscle deficits, genetic muscle fiber type ratio, pain intensity and apprehension level, etc.) that need to be considered when randomizing patients into groups. Combining groups by all potential confounding factors is virtually impossible in a clinical setting, thus differences between groups in some of the characteristics may have interfered with our results. Secondly, the extent of ischemic-reperfusion injury of QF muscle cannot be precisely quantified in the absence of humoral or tissue markers. It must be emphasized, however, that accurate estimation of small-scale ischemic-reperfusion injury of the QF muscle after ACL reconstruction is methodologically very demanding as inflammatory factors and markers of muscle cell injury released by ischemic-reperfusion injury are heavily masked by concomitant release of high concentrations of overlapping markers of tissue damage elicited by surgery *per se*. Thirdly, the increased muscle blood flow in the operated leg could be also influenced by postoperative inflammation of the knee joint and surrounding soft tissues. This seems, however, very unlikely, as no swelling, redness or increased temperature was noted in any of our patients at the site of measurement; the NIRS optode was placed at least 15 cm proximal to the affected joint, which is outside the wider inflammation area. Fourthly, the assessment of training gains in muscle function immediately after the BFR exercise intervention is lacking. To maximize the protective effect of IPC, last exercise unit had to be scheduled 24–48 h before the surgery, which made the in-between testing neither scientifically justified nor logistically feasible. The immediate effects of the preconditioning intervention thus remain unknown. Lastly, the regression models reported in the present study lack robustness as they are built on rather small number of observations.

Evaluation of molecular mechanisms of vasodilatory or vascular adaptation in the muscle following BFR exercise need to be further investigated. For more certain exclusion of the influences of inflammation on muscle blood flow, especially in the early postoperative period, capillary network density and presence of tissue inflammatory factors should be evaluated from muscle biopsies. Both peripheral and central neural mechanisms of enhanced muscle activation must be scrutinized in depth, with emphasis on modulation of pain and exertion thresholds during exercise. Future research should focus also on optimizing the protocol of preconditioning with low-load BFR exercise and timing of its application before surgery.

Physiological Relevance and Clinical Implications

This is the first study to demonstrate that short-term preconditioning with low-load BFR exercise training has significantly stronger positive effect on QF muscle endurance, its activation and perfusion after ACL reconstruction than equal training performed without blood flow restriction. However, both training regimes failed to affect the deterioration of maximal QF muscle strength following surgery. The preserved endurance of QF muscle was paralleled with enhanced muscle perfusion and increased amplitude of EMG for a given torque output. The latter corroborates the paradigm of enhanced volitional drive to the working muscles after IPC observed in athletes (Cruz et al., 2017). Given that some individuals with reconstructed ACL can have chronic 10% deficit in central activation ratio and 30% lower maximal EMG amplitude of QF muscle (Pamukoff et al., 2017), even small improvements in central motor drive induced by low-load BFR exercise would greatly increase QF muscle performance in these patients.

The importance of muscle perfusion in maintaining local muscle endurance after ACL reconstruction is a novel finding, which is in line with similar effects on muscle oxygenation and hemodynamics seen after low-load BFR training (Patterson and Ferguson, 2010; Kacin and Strazar, 2011) and IPC application (Jones et al., 2014, 2015; Jeffries et al., 2018) in healthy subjects. Based on the present results we presume that enhanced muscle perfusion after BFR training was a result of attenuated degradation of capillary network and preserved microcirculatory function in the atrophied muscle. Given that pre-operative muscle endurance has been shown to be the strongest independent predictor of QF muscle weakness following ACL reconstruction (Grapar Žargi et al., 2017), the preconditioning with low-load BFR exercise may serve as a valuable addition to standard physiotherapy programs for patients elected for ACL reconstruction, or other limb surgery performed with a tourniquet.

AUTHOR CONTRIBUTIONS

Conceived and designed the research, Interpreted the experimental results, and Approved the final version of the manuscript: TGŽ, MD, KS, and AK; Performed the experiments, Analyzed the data, and Drafted the manuscript: TGŽ and AK; Prepared the figures: AK; Edited and revised the manuscript: TGŽ, MD, and AK.

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Low-Load Resistance Training With Blood Flow Restriction Improves Clinical Outcomes in Musculoskeletal Rehabilitation: A Single-Blind Randomized Controlled Trial

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Background: There is growing evidence to support the use of low-load blood flow restriction (LL-BFR) exercise in musculoskeletal rehabilitation.

Purpose: The purpose of this study was to evaluate the efficacy and feasibility of low-load blood flow restricted (LL-BFR) training versus conventional high mechanical load resistance training (RT) on the clinical outcomes of patient's undergoing inpatient multidisciplinary team (MDT) rehabilitation.

Study design: A single-blind randomized controlled study.

Methods: Twenty-eight lower-limb injured adults completed a 3-week intensive MDT rehabilitation program. Participants were randomly allocated into a conventional RT (3-days/week) or twice-daily LL-BFR training group. Outcome measurements were taken at baseline and 3-weeks and included quadriceps and total thigh muscle cross-sectional area (CSA) and volume, muscle strength [five repetition maximum (RM) leg press and knee extension test, isometric hip extension], pain and physical function measures (Y-balance test, multistage locomotion test—MSLT).

Results: A two-way repeated measures analysis of variance revealed no significant differences between groups for any outcome measure post-intervention ($p > 0.05$). Both groups showed significant improvements in mean scores for muscle CSA/volume, 5-RM leg press, and 5-RM knee extension ($p < 0.01$) after treatment. LL-BFR group participants also demonstrated significant improvements in MSLT and Y-balance scores ($p < 0.01$). The Pain scores during training reduced significantly over time in the LL-BFR group ($p = 0.024$), with no adverse events reported during the study.

Conclusion: Comparable improvements in muscle strength and hypertrophy were shown in LL-BFR and conventional training groups following in-patient rehabilitation. The LL-BFR group also achieved significant improvements in functional capacity. LL-BFR training is a rehabilitation tool that has the potential to induce positive

adaptations in the absence of high mechanical loads and therefore could be considered a treatment option for patients suffering significant functional deficits for whom conventional loaded RT is contraindicated.

Trial Registration: ISRCTN Reference: ISRCTN63585315, dated 25 April 2017.

Keywords: blood flow restriction, musculoskeletal rehabilitation, hypertrophy, strength, function, pain, clinical outcomes

INTRODUCTION

Functional ability during rehabilitation is closely associated with improvements in strength training (Kristensen and Franklyn-Miller, 2012). Therefore, optimizing the potential for adaptations in muscle strength is an important consideration in the progression of any musculoskeletal (MSK) rehabilitation program. It is widely accepted within both the exercise science and rehabilitation medicine domains that to elicit significant gains in muscle strength and hypertrophy requires lifting loads $\geq$70% of an individual's 1-repetition maximum (1-RM) for a given movement (American College of Sports Medicine, 2009; Garber et al., 2011). However, for patients undergoing MSK rehabilitation, heavy-load resistance training (RT) can be contraindicated (Slysz et al., 2016) due to pain, muscle weakness and functional limitations preventing the attainment of these recommended heavier-loads (Hoyt et al., 2015). Patients with MSK injuries are often requested by their therapist to reduce the training load, potentially limiting the desired neuromuscular response to treatment and delaying the attainment of rehabilitation goals.

Blood flow restriction (BFR) exercise at low-loads (20–40% 1-RM) has been shown to be a safe (Loenneke et al., 2011) and effective tool to enhance the morphology and strength response in human muscle tissue (Slysz et al., 2016). However, the precise mechanisms underpinning the beneficial effects of BFR on skeletal muscle are unclear (Scott et al., 2015). A recent review reveals superior increases in muscle strength from heavy-load RT compared to low-load training with BFR, but comparable changes in muscular hypertrophy (Lixandrao et al., 2017). Low-load RT to volitional fatigue with and without BFR has demonstrated improvements in lower-limb muscle hypertrophy and endurance (Fahs et al., 2015). However, low-load RT with BFR was able to facilitate these improvements in muscle function using a reduced exercise volume (Fahs et al., 2015). There is now growing evidence for the practical and beneficial use of low-load blood flow restriction (LL-BFR) training as a clinical MSK rehabilitation tool (Takarada et al., 2000; Segal N. et al., 2015; Segal N.A. et al., 2015; Bryk et al., 2016; Giles et al., 2017; Tennent et al., 2017).

The majority of injuries in military populations occur in the lower limb (Anderson et al., 2016). There is subsequently a considerable economic and operational cost to the UK Ministry of Defence associated with lower-limb MSK injury. The Centre for Lower-Limb Rehabilitation at the UK Defence Medical Rehabilitation Centre (DMRC), Headley Court routinely treats and manages a large variety of lower-limb MSK disorders through 3 weeks multidisciplinary team (MDT) inpatient admissions. These injuries include, but are not limited to, overuse injuries (e.g., exertional lower-limb pain, patellofemoral pain, tendinopathy, and early osteoarthritis), bone fractures, post-surgical injuries (e.g., soft-tissue and ligamentous reconstruction) and hip and groin pain.

The development and investigation of emerging techniques with the potential to reduce recovery time and improve clinical outcomes is essential. To our knowledge, no studies have investigated the use of BFR exercise in a clinical population undergoing inpatient rehabilitation. Prior to the integration of novel techniques into clinical practice it is important to test the efficacy and safety against existing conventional training and rehabilitation methods. Therefore, we aimed to compare the effects of LL-BFR training with conventional heavy-load RT on changes in muscle volume and cross-sectional area (CSA), muscle strength and functional capacity in adults undergoing MSK inpatient rehabilitation. We also assessed the feasibility and adverse events associated with implementing LL-BFR exercise in a busy MDT rehabilitation setting.

MATERIALS AND METHODS

A detailed description of the study protocol including outcome measurement techniques and inclusion and exclusion criteria are published elsewhere (Ladlow et al., 2017). A description of the generic treatment pathway can be found in the **Supplementary File**.

Trial Design

This is a parallel group, two-arm, assessor-blinded randomized controlled trial (RCT) with a two (group), by two (time) repeated measures design. The RCT was registered with ISRCTN Registry, trial number 63585315 and data collection occurred from August 2016 to February 2017. Ethical approval was provided by the UK Ministry of Defence research ethics committee (reference protocol number: 442/MODREC/13). Participants provided written informed consent and were randomly allocated to a conventional high-load RT or LL-BFR training groups. Outcome measurements were assessed at baseline and 3-weeks.

This study has been designed and reported in line with the CONSORT recommendations for reporting randomized trials (**Figure 1**).

Participants

A heterogeneous group of 28 lower-limb injured male participants aged 19–49 years admitted for treatment at a MDT inpatient rehabilitation setting were recruited into the

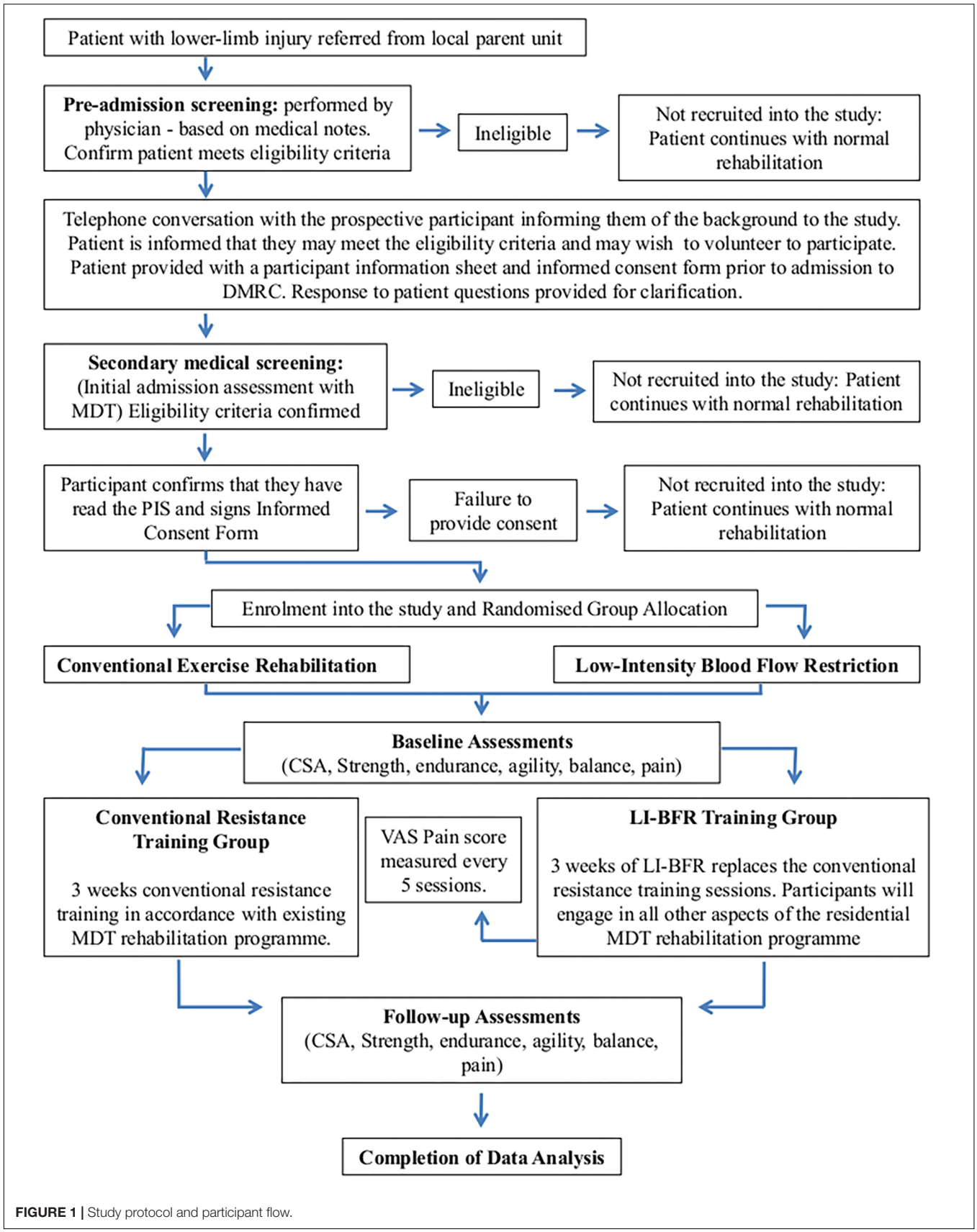

FIGURE 1 | Study protocol and participant flow.

study. Typically these lower-limb injured participants present with a functional status enabling load-bearing RT but not at a level to allow a return to work. See **Table 1** for the study inclusion/exclusion criteria and **Table 2** for participant injury diagnosis.

Randomization, Blinding and Screening Process

Eligible participants were randomly allocated into either an LL-BFR or conventional training group using blocked randomization at a 1:1 ratio. Clinicians responsible for recording study outcome measures and magnetic resonance imaging (MRI) imaging were blinded to participant group allocation. All patients then engaged in additional activities associated with their MDT inpatient admission. The screening process involved the patient attending a physician led MD injury assessment clinical 4 weeks prior to admission for MDT inpatient treatment. The screening comprised of a standard assessment of the patients history, clinical assessments appropriate for the diagnosis, imaging and x-ray where available in accordance with the MOD best-practice care pathway for lower-limb MSK injury. If following this screening the patient met the eligibility criteria for entry into the study they were approached to provide written informed consent.

TABLE 1 | Inclusion and exclusion criteria.

Inclusion criteria
1. Male
2. Between 18 and 50 years of age
3. Serving regular UK Armed Forces personnel
4. Lower limb injury (patellofemoral pain, ACL reconstruction, ankle injury, projectile/blast related injury)
5. Referred to Defence Medical Rehabilitation Centre (DMRC), Headley Court for treatment
6. Engaged in a minimum of 4 weeks exercise rehabilitation at their local primary health care facility (PCRF) or regional rehabilitation centre (RRU)
7. Present with a level of function that would enable them to engage in load bearing conventional exercise rehabilitation
8. Unable to return to active duty due to persistent pain or muscular dysfunction
Exclusion criteria
1. Female
2. Prior history of cardiovascular disease (hypertension, peripheral vascular disease, thrombosis/embolism, ischaemic heart disease, myocardial infarction)
3. Have a personal history of the following musculoskeletal disorders: rheumatoid arthritis, avascular necrosis or osteonecrosis, severe osteoarthritis
4. Have a personal history of the following neurological disorders: peripheral neuropathy, Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, stroke, mild or severe traumatic brain injury
5. Have chronic or relapsing/remitting gastrointestinal disorders such as inflammatory bowel diseases, irritable bowel syndrome or gastrointestinal infections within 28 days of screening
6. Have an acute viral or bacterial upper or lower respiratory infection at screening
7. Have moderate or severe chronic obstructive pulmonary disease (COPD)
8. Amputation to the lower or upper extremity
9. ACL surgery within the last 4 weeks
10. Surgical insertion of metal components in lower limbs (may affect MRI results)
11. Have a personal history of any of the following conditions or disorders not previously listed: diabetes, fibromyalgia, active cancer, severe obesity (i.e., body mass index greater than 35 kg/m^2), diagnosed mental illness (e.g., PTSD, depression, anxiety)
12. Have a current or previous use of any drugs known to influence muscle mass or performance within previous 6 months
13. Yet to receive any formal progressive exercise rehabilitation treatment within the past 4 weeks
14. Participants were excluded from the study if they were found to be at elevated risk of unexplained fainting or dizzy spells during physical activity/exercise that causes loss of balance

TABLE 2 | Descriptive characteristics, injury diagnosis, muscle CSA/volume, strength measurements and functional performance status recorded at baseline.

Baseline measurements	LL-BFR	Conventional RT
Participant characteristic (m ± *SD*)		
Participant numbers	14	14
Age (years)	33 ± 6	28 ± 7
Body height (cm)	178 ± 6	179 ± 7
Body mass (kg)	88 ± 19	92 ± 13
Body mass index (kg m^2)	28 ± 5	29 ± 3
Diagnosis, number (%)		
Exertional lower limb pain	6 (43)	6 (43)
Patellofemoral pain syndrome	3 (21)	1 (7)
Knee Surgery (e.g., ligament reconstruction)	2 (14)	3 (21)
Hip injury/surgery (e.g., arthroscopy)	2 (14)	3 (21)
Other lower-limb injury	1 (7)	1 (7)
Bilateral symptoms	9 (64)	8 (57)
Baseline test performance scores (m ± *SD*)		
Quadriceps muscle CSA (cm^2)*	90 ± 17	95 ± 14
Quadriceps muscle volume (cm^3)*	2207 ± 486	2283 ± 400
Thigh muscle CSA (cm^2)*	200 ± 34	209 ± 27
Thigh muscle Volume (cm^3)*	5278 ± 1123	5330 ± 848
Leg-press 5-RM (kg)*	78 ± 28	89 ± 33
Knee-extension 5-RM (kg)*	27 ± 14	30 ± 11
Isometric hip extension (N)*	265 ± 84	298 ± 87
Endurance (MSLT) (m)	1057 ± 461	1137 ± 600
Pooled Y-balance test (cm)*	264 ± 29	280 ± 25

**Data reflects the injured limb only. LL-BFR, low-load blood flow restriction; RT, Resistance Training; CSA, cross-sectional area; RM, Repetition maximum; MSLT, multi-stage locomotion test.*

Assessment Procedures

Determining Arterial Occlusion Pressure

The participant's limb occlusion pressure (LOP) was determined prior to commencing the LL-BFR training program with the patient lying flat in a supine position. A 10-cm wide blood pressure cuff (Schuco TourniCuff, Schuco International, Watford, United Kingdom) was placed around the most proximal part of each thigh. The posterior tibial or dorsalis pedis pulse was located with a MD2 vascular doppler probe (Huntleigh Healthcare Ltd., Cardiff, United Kingdom). The tourniquet was rapidly inflated using a PTSii portable tourniquet system (Delfi Medical Innovations, Vancouver, BC, Canada) to a pressure of 250 mmHg (Noordin et al., 2009) such that the audible pulse was lost and then deflated until the pulse was regained; 60% of this

LOP was calculated and used as the tourniquet pressure during the LL-BFR intervention (Scott et al., 2015).

Feasibility and Acceptability of LL-BFR Intervention

Acceptability was assessed by examining reasons for drop-out in any discontinuing participants and by comparing attrition rates between groups. Strengths, weaknesses and safety of the LL-BFR intervention was assessed by qualitative interviews with the project supervisor, lead exercise rehabilitation instructor, participant feedback and examination of adherence rates and adverse event reports.

Outcome Measures

All outcome measures were assessed at baseline and upon completion of 3-weeks inpatient rehabilitation using standardized and validated tests.

Muscle Hypertrophy

Muscle CSA and Volume

For each slice of the injured limb, quadriceps and hamstring muscle compartments CSA (cm^2) were measured and muscle compartment volumes calculated (cm^3). Thigh CSA and volume encompassed both quadriceps and hamstring muscle architecture. Measurements were assessed prior to and 24-h following the completion of the 3-weeks rehabilitation program, using MRI with a GE Sigma scanner 1.5T (General Electric, WI, United States) in accordance with the method previously described by Abe et al. (2003). The same assessor completed baseline and post-intervention scans. All participants had both legs scanned with only the injured limb used for analysis purposes.

Muscle Strength

5-RM Knee Extension and Leg Press

Unilateral muscle strength was assessed using a dynamic 5-RM knee extension and a 45° incline leg press test (Pulse Fitness, Congleton, United Kingdom). An initial resistance was set based upon the result of a clinical assessment, pain intensity and participant feedback. The resistance was adjusted and test repeated until the participant was unable to complete five-repetitions. Participants received 3-min rest between each attempt and were allowed a maximum of three attempts to produce a 5-RM. This procedure followed established and widely used guidelines (Baechle and Earle, 2008).

Isometric Hip Extension

Unilateral isometric hip extension strength was assessed using a wireless digital microFET2 hand-held dynamometer (Hoggan Scientific LLC, Drapper, UT, United States) by the same assessor at baseline and post-intervention. The participant exerted a 5-s isometric maximal voluntary contraction (MVC) against the dynamometer and the examiner, whilst lying prone on a clinical examination couch as recommended by Thorborg et al. (2010). Participants performed four consecutive attempts with a 30-s recovery between attempts. Measures were reported as Newtons (N) with the highest value used for analysis.

Endurance

Endurance was measured using the multistage locomotion test (MSLT). The objective of this test was to assess the participant's maximal walk/run distance (Vitale et al., 1997; Hassett et al., 2007). The test required the participant to walk/run on a 20-m track at gradually increasing speeds until they were unable to continue. Speed was controlled by paced-auditory cues accompanied by recorded verbal instructions. The test was terminated when the participant failed three consecutive attempts to reach the designated marker on the audible cue. Total distance covered in meters was recorded.

Balance

The Y-balance test assesses lower-body balance and flexibility using the Y-balance test kit (Plisky et al., 2009). Standing through a single supporting limb on the test kit, the participant reached with the free limb as far as possible along three lines positioned in anterior, postero-medial, and postero-lateral directions on each leg. To gain a global indicator of dynamic posture and balance, pooled data from all movement planes were calculated (distance performed in cm) and used for analysis.

Pain

A 100 mm horizontal visual analog scale (VAS) was used to measure pain and physical discomfort every five LL-BFR treatment sessions over the 3 weeks intervention (Collins et al., 1997). Using the VAS instrument, participants were asked "How do you rate the level of physical discomfort associated with the LL-BFR exercise," immediately prior to starting the exercise, during the exercise and 5 min post-exercise. Symptomatic pain (the reproduction of pain at the associated site of injury) during LL-BFR was also assessed.

Treatment Procedures

Before embarking on a fully powered RCT we wanted to assess the feasibility of BFR training against traditional RT methods employed in MSK rehabilitation. The primary aim of this RCT was to assess whether LL-BFR training is a rehabilitation tool that has the potential to induce positive adaptations in the absence of high mechanical loads (i.e., conventional RT). Therefore, we purposely selected a low-load non-weight bearing protocol in combination with BFR versus a traditional high mechanical load weight bearing protocol in our study to address the research question and properly assess the utility of BFR in our lower-limb injured patients. All patients recruited would have been functionally able to complete either intervention group. However, based on the exercises selected, the LL-BFR training protocol does not require upright mechanical loading whereas the conventional RT protocol does. To select two identical exercise protocols would not have addressed this fundamental question and is at the essence of this proof of concept RCT.

LL-BFR Training

A 10-cm wide contoured blood pressure cuff was placed around the proximal end of each thigh and inflated to the pre-determined 60% LOP. Participants performed low-load RT (30% 1-RM) combined with BFR using two exercises in sequence: (1) bilateral

leg press using a leg press machine (Pulse Fitness, Congleton, United Kingdom), and (2) bilateral knee extensions using a knee extension machine (Pulse Fitness, Congleton, United Kingdom). 30% of 1-RM was determined based on an estimated 1-RM using their 5-RM muscle strength assessments. These exercises (one open chain quadriceps exercise and one closed chain with contributions from quadriceps and hip extensors muscles) enable RT to be performed with reduced axial loading. Off-loading an injured limb, whilst simultaneously provoking muscular overload is an essential component in the progression of many MSK rehabilitation programs. When full-loading bearing is not advised or contraindicated, these two exercises can be considered a suitable alternative (to traditional squatting, lunging, or deadlifts), and are frequently prescribed together in the prescription of lower-limb BFR training (Karabulut et al., 2010; Shimizu et al., 2016). Participants performed four sets of 30, 15, 15, and 15 repetitions at 30% of their predicted 1-RM (Segal N. et al., 2015; Segal N.A. et al., 2015; Giles et al., 2017; Tennent et al., 2017), with an inter-set interval of 30-s. A gradual progression of load lifted over the intervention period was permitted but based on patient feedback and clinician discretion. The inflation pressure was maintained for the duration of the exercise component and deflated during the 3-min inter-exercise rest interval.

The total length of time exposed to restricted blood flow was 4-min per exercise and 8-min per training session. Training was performed twice daily in the morning and afternoon (always separated by interludes of $\geq$5 h), from Monday to Thursday and once on Friday morning.

Conventional (High-Load) RT

Participants completing conventional RT performed four-sets of three-exercises (deadlift, back squat, and lunges) three times per week. A gradual exercise progression using these closed chain exercises was determined by the exercise rehabilitation instructor based upon individual response to training. Repetitions per set were typically 6–8 and tailored to the individual needs of the patient with 3-min rest intervals between each set. The load lifted was a reflection of their best effort taking into account each individual's injury limitations (for example, pain inhibition or inability to provide sufficient force due to weakness associated with their traumatized joint or muscle tissue). This protocol represents the type of exercise unavailable to patients suffering higher pain scores and lower levels of function.

Over the 15-days of supervised MDT rehabilitation participants completed a maximum of 23 8-min LL-BFR training sessions or 9 1-h conventional RT sessions. A full description of the standard 3-weeks MDT program, LL-BFR exercises, outcome measurement technique and example MRI images are provided in an online **Supplementary File**.

Sample Size

No formal sample size calculation determined by statistical assumptions and tests was performed as this was a pilot study design. Sample size recommendations for pilot RCTs were followed (Julious, 2005). Given the time constraints for data collection for this study we used a convenient sample with 14 participants recruited into each treatment group.

Statistical Analysis

Results are presented using mean, SD and percentage change over time. Descriptive statistics were used to summarize eligibility, consent, randomization, adverse events, retention, completion, and intervention adherence rates. Participant demographic and baseline characteristics were also compared and reported. The results of strength, function and muscle volume/CSA tests were analyzed to evaluate group differences using a two-way repeated measures (time $\times$ group) analysis of variance (ANOVA). Even though there were no statistical differences between groups at baseline, analysis of covariance (ANCOVA) was used on muscle CSA, volume, strength and functional measurements to correct for any baseline differences and an adjusted post-intervention and change score reported as recommended by (Vickers and Altman, 2001). This statistical analysis of the data was exploratory only as our sample size did not allow for a definitive analysis. The level of significance was set at $p < 0.05$. All analysis was carried out using SPSS v.22.0.

RESULTS

Baseline Data

Table 2 summarizes the baseline demographic, diagnostic injury characteristics, muscle CSA/volume, muscle strength, and functional performance outcomes by group.

Limb Occlusion Pressure

LL-BFR group participants had bi-lateral LOP measured ($n = 28$ limbs) before training commenced. After calculating 60% LOP, individualized tourniquet pressures ranged between 105 and 144 mmHg (mean: 124 $\pm$ 13 mmHg).

Between Group Changes Over Time for All Outcomes

A two-way repeated measures ANOVA demonstrated no significant differences between groups for any outcome measure ($p > 0.05$). However, after adjusting for differences in baseline values there was a significant difference in mean quadriceps muscle volume [$F(1,42) = 10.371, p = 0.002$] after 3-weeks between LL-BFR and conventional RT group.

Within Group Changes Over Time for All Outcomes

Muscle CSA and Volume

A total of 45 injured limb (23 LL-BFR; 22 conventional RT group—some patients from each group presented with bilateral injuries) scores were analyzed. At 3-weeks both groups had significantly increased their quadriceps and thigh CSA and volume in the injured limb ($p < 0.01$). **Figure 2** shows quadriceps CSA increased 7 and 5%; quadriceps volume 8 and 3%; thigh CSA 4 and 5%; thigh volume 3 and 4% in the LL-BFR and conventional RT groups, respectively. After adjusting for baseline values,

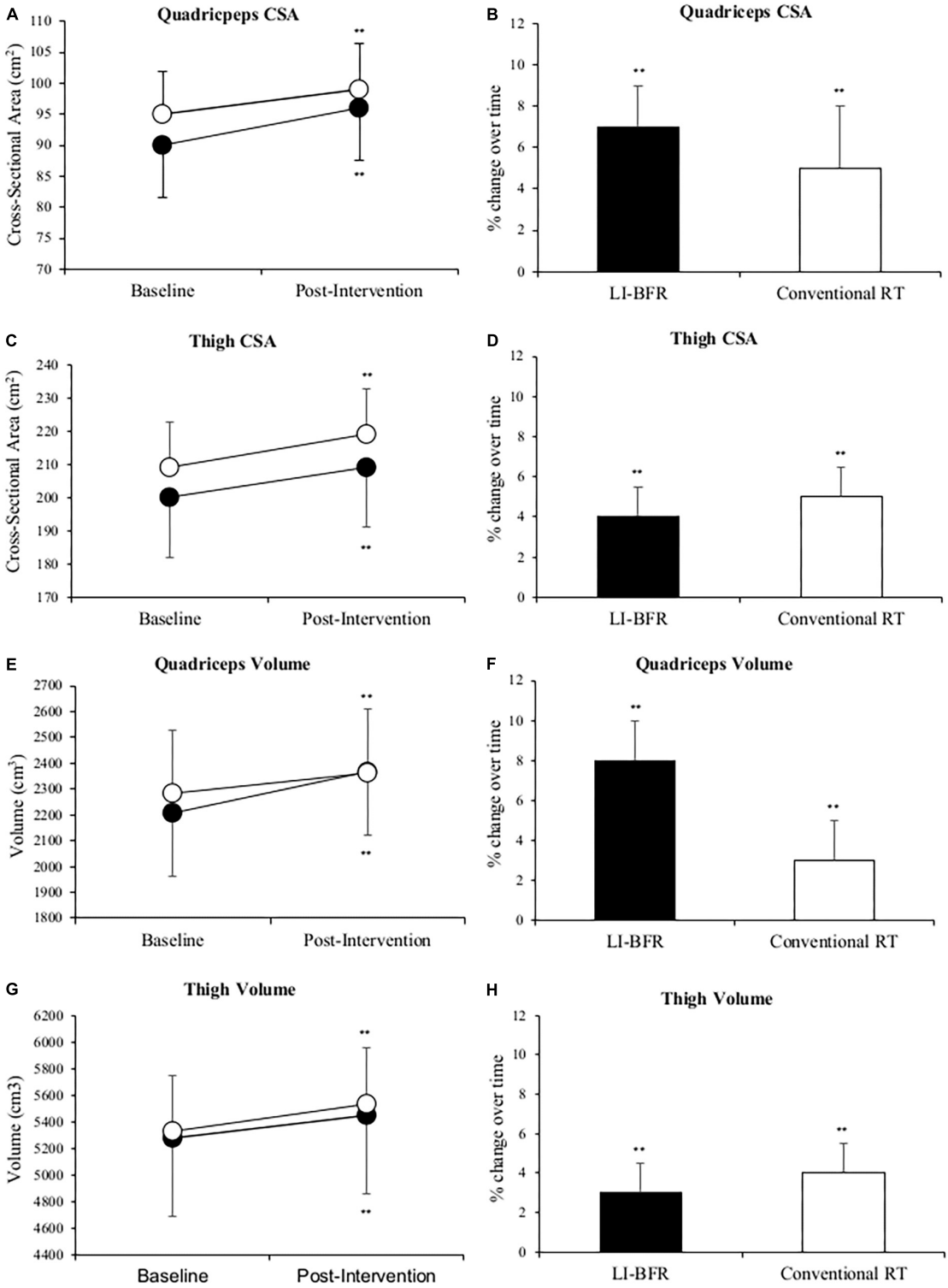

FIGURE 2 | Changes in quadriceps muscle cross-sectional area (CSA) **(A,B)**, thigh CSA **(C,D)**, quadriceps muscle volume **(E,F)** and thigh muscle volume **(G,H)** at baseline and after 3-weeks of rehabilitation. Black points LL-BFR group, White points Conventional Resistance Training (RT) group. Bar charts show group percent changes over time. Data is expressed as mean ± SD. * $p < 0.05$ and ** $p < 0.01$ versus baseline measurement. Data refers to primary injured limb. LL-BFR, low-load blood flow restriction; RT, resistance training; CSA, cross-sectional area.

TABLE 3 | Post-intervention values and change score of muscle cross-sectional area (CSA) and muscle volume after adjusted for differences in values scored at baseline (using analysis of covariance—ANCOVA).

	Adjusted pre-intervention value (m)	Intervention	Mean adjusted change score (95% CI)	Mean between group differences (95% CI)
Quadriceps muscle CSA (cm^2)	92	LL-BFR	6 (4–8)	2 (−1 to 4)
		Conventional RT	4 (2–6)	
Quadriceps muscle volume (cm^3)	2244	LL-BFR	160 (125–196)	82 (31–133)
		Conventional RT	79 (42–115)	
Thigh muscle CSA (cm^2)	205	LL-BFR	9 (6–12)	1 (−3 to 5)
		Conventional RT	10 (7–13)	
Thigh muscle volume (cm^3)	5303	LL-BFR	172 (109–234)	34 (−56 to 124)
		Conventional RT	206 (142–270)	

Data reflects the injured limb only. Data presented as mean ± SE and 95% confidence intervals (CIs). LL-BFR, low-load blood flow restriction; RT, resistance training; CSA, cross-sectional area.

the adjusted change score between groups were comparable in CSA values, however, the LL-BFR group demonstrated a greater change score in quadriceps volume whilst the conventional RT group demonstrated a greater change score in thigh muscle volume (**Table 3**).

Lower-Limb Muscle Strength

Figure 3 shows that mean 5-RM leg press and knee extension performance in the injured limb significantly improved in both groups ($p < 0.01$). Leg press strength improved 16 and 25%, knee extension strength improved 40 and 24% in the LL-BFR and conventional RT groups, respectively. Although positive changes in mean isometric hip extension strength (23 ± 66 N) were reported in the LL-BFR group but not in the conventional RT group (−17 ± 75 N), no significant changes occurred over time in either group ($p > 0.05$). After adjusting for baseline values, the conventional RT group demonstrated a greater mean change score in 5-RM leg press and the LL-BFR group demonstrated a greater mean change score in 5-RM knee extension (**Table 4**).

Functional Outcomes

Mean MSLT distance significantly improved by 29% (306 ± 246 m, $p = 0.01$) in the LL-BFR group. The conventional RT group also recorded a greater mean distance covered after treatment (91 ± 341 m) but this change was non-significant ($p > 0.05$). LL-BFR group participants demonstrated a significant improvement (15 ± 20 cm, $p = 0.03$) in pooled Y-balance test scores, whereas conventional RT participant scores (−1 ± 32 cm, $p = 0.93$) did not improve (**Figure 4**).

Compliance/Acceptability/Feasibility and Pain Response to LL-BFR Exercise

Full patient compliance and adherence to the twice daily LL-BFR intervention was demonstrated. Mild muscular discomfort during exercise was reported (**Figure 5**) with self-reported pain returning to pre-exercise levels 5-min post-exercise. Mean symptomatic pain scores did not significantly change throughout the intervention (range: 13–19 mm). Pain reported during LL-BFR training was significantly greater (range: 44–66 mm) than pain reported before and after exercise ($p < 0.01$). When compared with baseline, there was a reduction in levels of muscular discomfort reported at commencement of the third week (day 10).

DISCUSSION

To our knowledge this is the first study using muscle volume and CSA, strength and functional capacity measures to demonstrate the application of LL-BFR when used as an adjunct to an inpatient musculoskeletal injury (MSKI) rehabilitation program. Both LL-BFR and conventional training groups showed significant within-group changes in muscle CSA/volume, 5-RM leg press and 5-RM knee extension scores after treatment. There were significant improvements in LL-BFR group participants MSLT and Y-balance test scores. The conventional training group functional capacity scores did not improve over time. Greater within-group changes and adjusted mean scores over time in the LL-BFR participants were observed; however, this does not constitute a superior training effect as the results of the two-way ANOVA showed no statistically significant differences between groups over time. Only after adjusting for baseline values was a significant difference between groups in quadriceps muscle volume found. Feasibility assessment revealed there were no drop-outs, no adverse events and full compliance associated with the LL-BFR intervention.

Effects on Muscle Strength and Hypertrophy

Increasing muscle strength is a crucial aim of rehabilitation for all MSKI as muscle weakness is associated with delayed recovery and functional impairment (Kristensen and Franklyn-Miller, 2012). The comparable changes in clinical outcomes between LL-BFR and conventional RT in this study support previous findings in MSK rehabilitation research (Segal N. et al., 2015; Segal N.A. et al., 2015; Giles et al., 2017). Although greater mean change scores and percentage increases in 5-RM knee extension, isometric hip extension strength were not significantly different between groups they may be clinically relevant (Giles et al., 2017). Specifically, comparison of perceptual pain and perceived exertion responses during LL-BFR (30% 1-RM) versus heavy

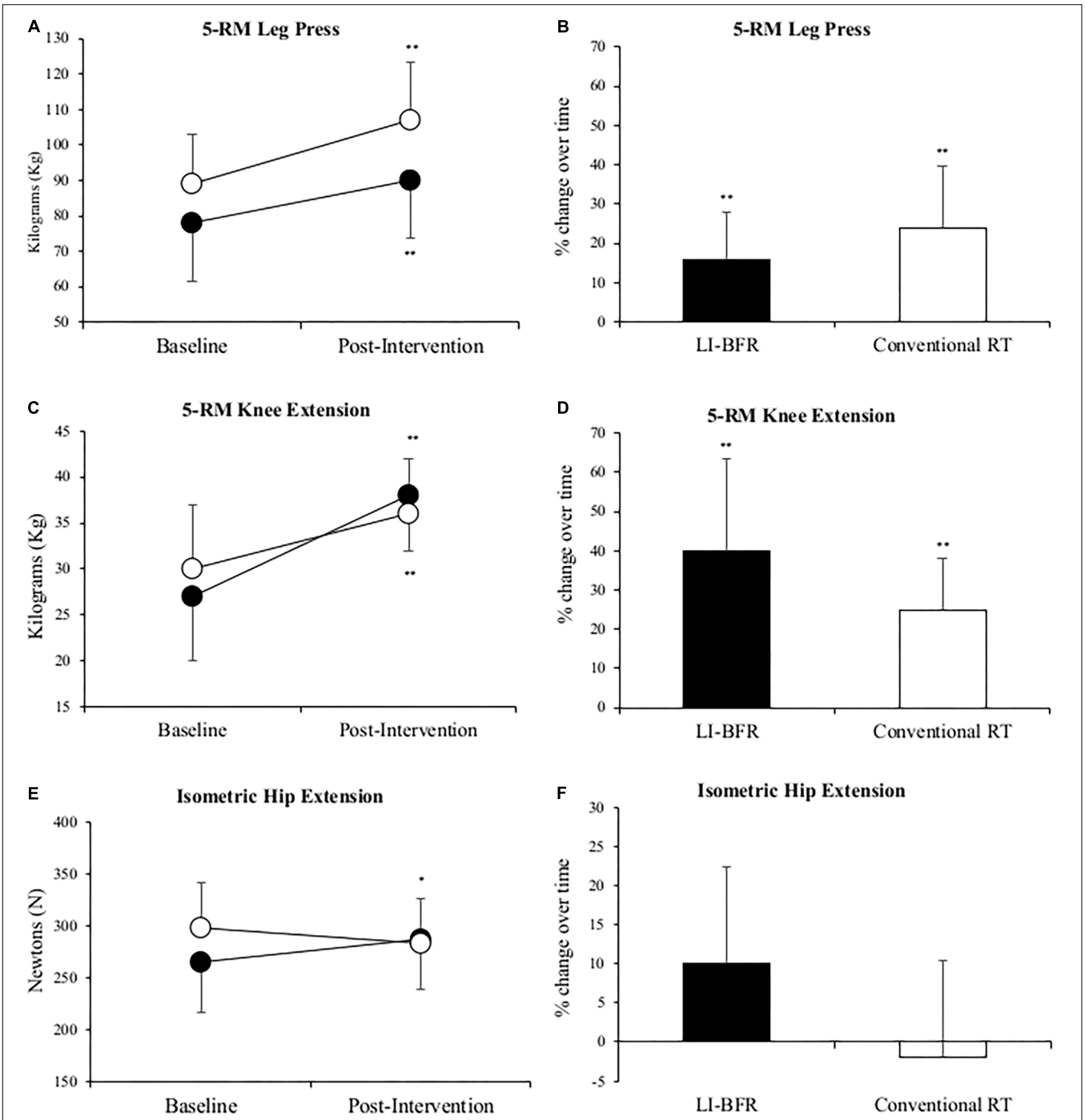

FIGURE 3 | Changes in 5-RM leg press **(A,B)**, 5-RM knee extension **(C,D)** and isometric hip extension **(E,F)** at baseline and after 3-weeks of rehabilitation. Black points LL-BFR group, White points Conventional Resistance Training (RT) group. Bar charts show group percent changes over time. Data is expressed as mean ± SD. *$p < 0.05$ and **$p < 0.01$ versus baseline measurement. Data refers to primary injured limb. LL-BFR, low-load blood flow restriction; RT, resistance training; RM, repetition maximum.

load (70% 1-RM) demonstrate these responses are generally lower in BFR groups compared with equivalent exercises at higher intensities (Hollander et al., 2010; Hughes et al., 2017; Giles et al., 2017). Therefore, the greater overall muscle strength gains in our BFR group may be due to lower joint forces and stress during BFR exercise allowing BFR participants to better tolerate these perceptual pain and exertion changes compared to the conventional training group. Whilst appealing, the current evidence-base supporting this position is limited (Hughes et al., 2017). However, Haun et al. (2017) found that muscle soreness

TABLE 4 | Post-intervention values and change score of muscle strength and functional test after adjusting for differences in values scored at baseline (using analysis of covariance—ANCOVA).

	Adjusted pre-intervention value (m)	Intervention	Mean adjusted change score (95% CI)	Mean between group differences (95% CI)
Leg press 5-RM (kg)	88	LL-BFR	12 (6–19)	4 (−5 to 14)
		Conventional RT	16 (9–23)	
Knee extension 5-RM (kg)	29	LL-BFR	9 (6–12)	3 (−1 to 7)
		Conventional RT	6 (3–8)	
Isometric hip extension (N)	281	LL-BFR	18 (−11 to 47)	35 (−7 to 78)
		Conventional RT	−17 (−48 to 13)	
MSLT (m)	1085	LL-BFR	306 (140–472)	215 (−25 to 455)
		Conventional RT	91 (−81 to 264)	
Pooled Y-balance test s(cm)	272	LL-BFR	12 (1–22)	11 (−5 to 26)
		Conventional RT	1 (−9 to 12)	

Data reflects the injured limb only. Data presented as mean ± SE and 95% confidence intervals (CIs). LL-BFR, low-load blood flow restriction; RT, resistance training; CSA, cross-sectional area; RM, repetition maximum; MSLT, multi-stage locomotion test.

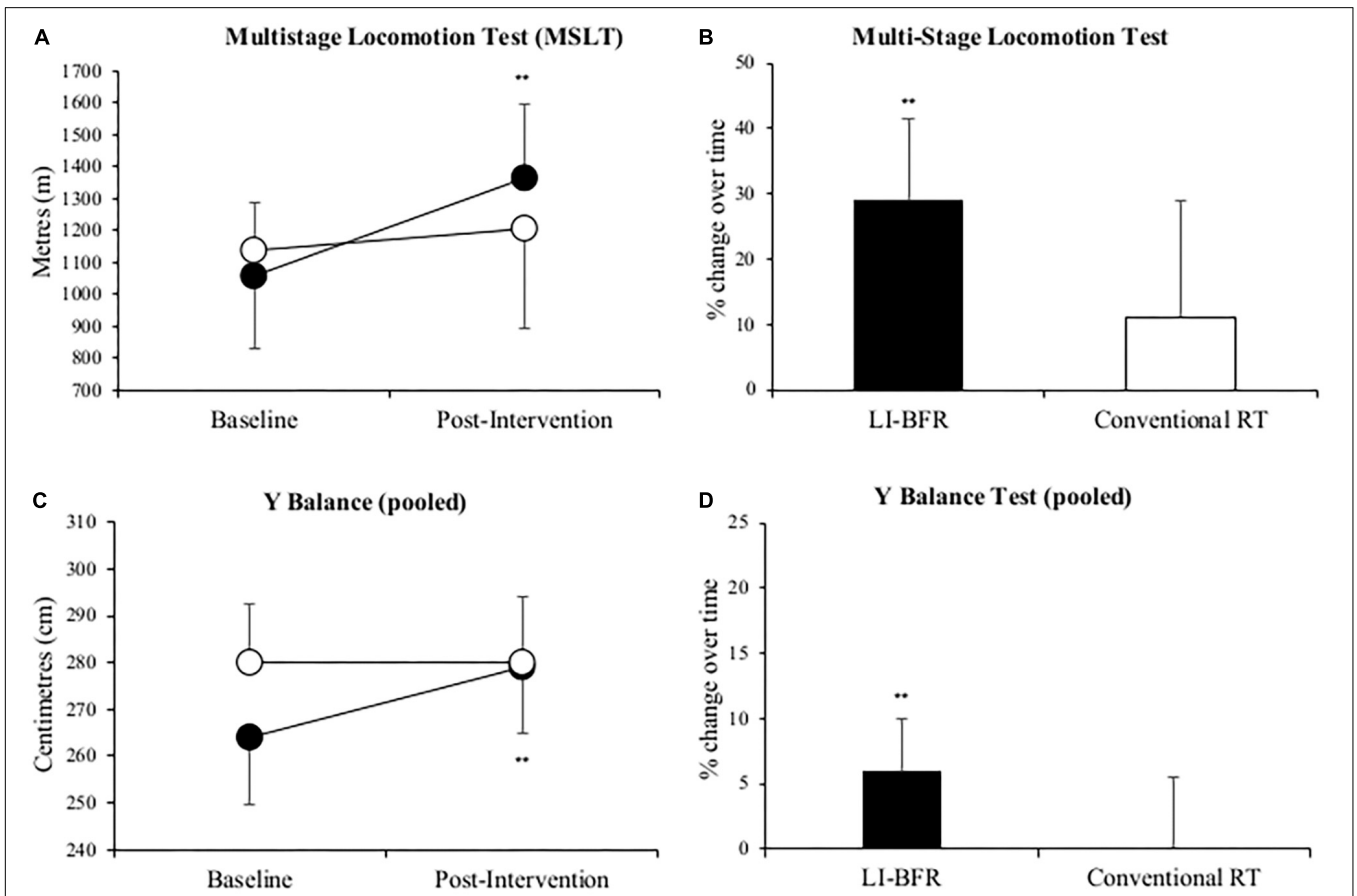

FIGURE 4 | Changes in multi-stage locomotion test (MSLT) **(A,B)**, and pooled Y balance **(C,D)** at baseline and after 3-weeks of rehabilitation. Black points LL-BFR group, White points Conventional Resistance Training (RT) group. Bar charts show group percent changes over time. Data is expressed as mean ± SD. * $p < 0.05$ and ** $p < 0.01$ versus baseline measurement. Data refers to primary injured limb. LL-BFR, low-load blood flow restriction; RT, resistance training.

reduced following post-exercise BFR, therefore BFR training could have a role in modulating pain.

The mean changes in quadriceps muscle CSA and volume (7 and 8%, respectively) in our study are comparable to those reported following a 12-days (twice daily) BFR-intervention in healthy subjects (Abe et al., 2005). A more recent RCT comparing conventional therapy with and without BFR after knee arthroscopy also reported greater hypertrophic gains (thigh girth at 6- and 16-cm proximal to patella pole) in a BFR treatment group (Tennent et al., 2017). Alongside our results,

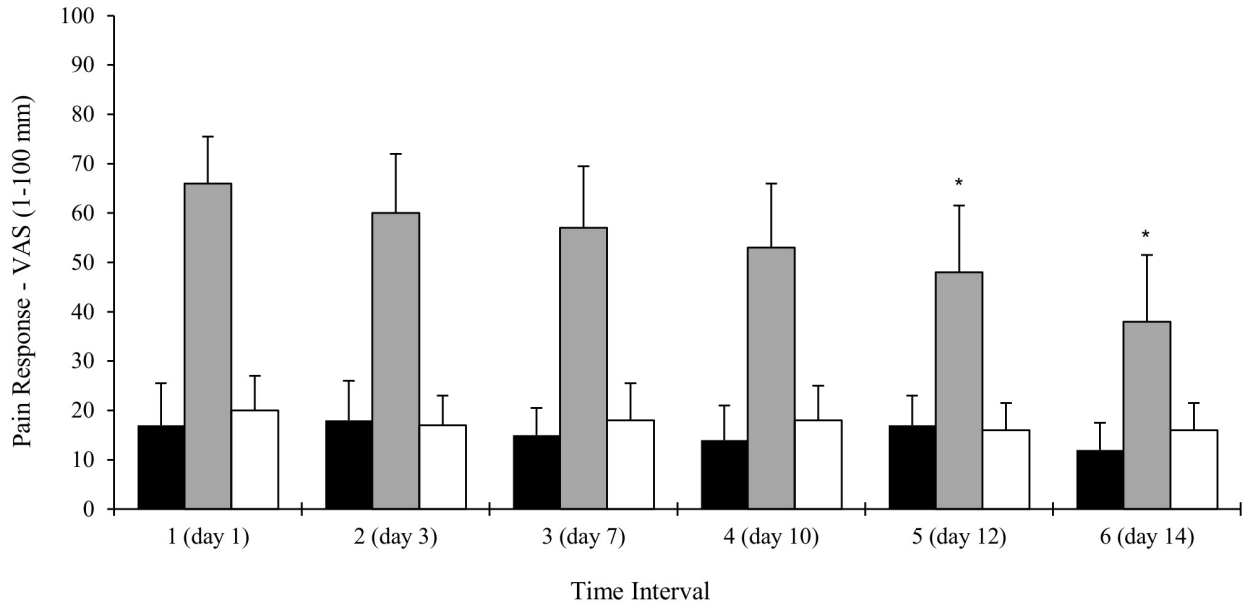

FIGURE 5 | Changes in LL-BFR participant's self-reported pain before, during and 5 min after the completion of exercise. Data collected over six time points (every five training sessions) during the 3-weeks intervention. Data is expressed as mean ± SD, $^*p < 0.05$. VAS, visual analog scale.

these findings demonstrate that short-term twice daily LL-BFR can result in significant hypertrophy adaptations in both healthy adults and lower-limb MSK injured patients. It is proposed that adaptations in muscle hypertrophy and strength are a result of metabolic stress associated with BFR and the mechanical tension of the load lifted acting synergistically to mediate numerous secondary mechanisms, all of which stimulate autocrine and/or paracrine actions to facilitate muscle growth (Pearson and Hussain, 2015).

Different mechanisms behind muscle hypertrophy and muscle strength in response to BFR training have been proposed (Jessee et al., 2018). The proposed mechanisms to elicit muscle hypertrophy include muscle cell swelling (Loenneke et al., 2012) and metabolite-induced fatigue (Abe et al., 2006). These mechanisms are considered to influence the intramuscular anabolic/anti-catabolic signaling response for protein synthesis (Fujita et al., 2007; Fry et al., 2010; Laurentino et al., 2012). The production of reactive oxygen species (ROS) (Kawada and Ishii, 2005; Pope et al., 2013) and post-exercise reductions in muscle oxidative stress and proteolysis (Haun et al., 2017) are also considered to influence muscle growth. The mechanisms behind strength adaptations and the role of the neuromuscular system are less clear. However, an acute bout of BFR training appears to increase corticomotor excitability (Brandner et al., 2015) and proposed to increase in fast twitch muscle fiber/motor unit recruitment (Takarada et al., 2002; Cook et al., 2013). However, a recent study by Hill et al. (2018) found no changes in muscle activation (EMG amplitude) or electrical efficiency during a 4 weeks BFR intervention and concluded that early phase increases in muscle strength are not associated with neural changes, but was likely a result of muscle hypertrophy. However, in the absence of research demonstrating a causal link, any suggested associations between BFR training and subsequent muscle growth are purely speculative.

Effects on Physical Function Outcomes

In MSK rehabilitation practice much emphasis is placed on the importance of physical function. However, very few studies have assessed this component of treatment in BFR research. One study has demonstrated greater improvements in a timed stair ascent task following conventional therapy with BFR in knee OA patients (Tennent et al., 2017). In our study significant improvements in endurance (MSLT distance) were demonstrated in the LL-BFR group participants. It has been reported that favorable adaptations occur within vascular networks as a response to LL-BFR (Casey et al., 2010; Hunt et al., 2013). Although these mechanisms were not tested, it is possible this may have contributed toward improved endurance capacity changes in the LL-BFR group. We also found a non-significant improvement in mean isometric hip extension strength in the LL-BFR group (23 N). Previous research has reported strength and hypertrophic adaptations in the muscles located proximal to the applied pressure as a result of pre-fatiguing in the muscles below the cuff (Dankel et al., 2016). This enhanced stimulation of the hip musculature (located proximal to the cuff) may explain the significant improvements demonstrated in pooled Y-balance scores in the LL-BFR group relative to the conventional RT group. Enhanced hip muscle strength may also contribute to improvements in walking/running mechanics and therefore endurance capacity. Further research is required to better understand how muscle adaptations to BFR exercise influences functional capacity in MSK injured populations.

Feasibility Components

Comparison of attrition rates between groups revealed no recorded drop-outs or any discontinuing participants from either group. Session adherence rates were 100% (LL-BFR) and >90% in the conventional training group. No adverse events or safety breaches were observed and LL-BFR participant mean pain scores during training reduced over time (**Figure 5**) suggesting a degree of adaptation to an unfamiliar exercise stimulus.

Limitations

Our participants were suffering MSK injury of the lower-limb at the same stage of functional recovery, however, they comprised a mix of diagnostic injury types undergoing a complex multi-modal intervention and some heterogeneity in clinical severity may have attenuated the treatment effect. We did not follow-up our participants beyond the 3-weeks period of rehabilitation and no conclusions can be made on any longer-term benefits of treatment. Due to time-limited constraints for data collection we used a convenient sample. The small sample size limits the ability to make definitive statements regarding the effectiveness of LL-BFR and results may be susceptible to type I or type II errors. Whilst the use of a young male population may limit the generalizability to other populations and settings, we believe the findings are relevant to any MSK injured rehabilitation population. The use of different loading conditions and exercises between groups was intentional to properly address the aims of the study; however, we acknowledge a specificity of training effect may have led to the greater change scores for 5-RM knee extension and quadriceps muscle volume in the LL-BFR group. Future research investigating BFR training should consider the potential for a specific transfer of strength gains between training and testing. Also, due to insufficient data in the conventional RT group, comparisons in exercise volume were not possible; this should be a consideration in any future BFR related study using clinical populations.

CONCLUSION

This is the first study to demonstrate that twice daily LL-BFR exercise at 30% 1-RM can be safely and effectively implemented into a busy inpatient MDT rehabilitation setting. Twice daily BFR training at low-load (30% 1-RM) resulted in significant improvements in lower-limb muscle hypertrophy, strength and function after 3-weeks inpatient rehabilitation. LL-BFR training yielded positive gains in participant physical function relative to conventional RT. Both conventional RT and LL-BFR can safely be used to improve clinical outcomes; however, LL-BFR training is a rehabilitation tool that has the potential to induce positive adaptations in the absence of high mechanical loads. This finding may have implications for patients suffering significant functional deficits for whom conventional training is contraindicated. Further studies using randomized designs examining the effects of LL-BFR training in patients with greater levels of impairment are needed.

AUTHOR CONTRIBUTIONS

PL, RC, SD-D, and SP conceived the study design. AB obtained the funding. SD-D and DC were responsible for recruiting and consenting participants into the study, and delivering the intervention. ES analyzed all MRI related outcomes. PL, RC, and ES analyzed the findings. PL and RC produced a draft manuscript. All authors read, critically reviewed, and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01269/full#supplementary-material

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Hemodynamic Responses to Low-Load Blood Flow Restriction and Unrestricted High-Load Resistance Exercise in Older Women

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Introduction: Blood flow restriction (BFR) during low-load resistance exercise increases muscle size similarly to high-load training, and may be an alternative to lifting heavy weights for older people at risk of sarcopenia. However, few studies have addressed the safety of such exercise in older people, or whether this is impacted by the actual exercises performed during training. This study aimed to compare the acute hemodynamic and perceptual responses during low-load BFR exercise to unrestricted low-load and high-load exercise in older women, and to determine whether these responses depend on the type of exercise performed.

Methods: Fifteen older women (63–75 year) were assessed for maximal strength (1RM) in the leg press and leg extension. Participants then completed three protocols using these exercises in a randomized order: (1) low-load exercise (LL); (2) low-load exercise with BFR (LL_{BFR}), and; (3) high-load exercise (HL). Blood pressure was assessed at baseline and after each set, and impedance cardiography measured cardiovascular function during trials. Rating of perceived exertion (RPE) and muscle soreness scores were obtained throughout trials.

Results: Baseline hemodynamic values were consistent between trials. Systolic, diastolic, and mean arterial pressures were higher in LL_{BFR} compared with HL and LL ($p \leq 0.021$). The LL condition resulted in lower heart rate ($p \leq 0.002$) and rate-pressure product ($p \leq 0.011$) responses compared with LL_{BFR} and HL. The leg press generally conferred greater hemodynamic and perceptual demands than the leg extension for all conditions ($p \leq 0.002$). RPE was lower during LL compared with LL_{BFR} and HL ($p \leq 0.008$), and there were no between-condition differences in perceived muscle soreness.

Conclusion: The blood pressure data indicate that LL_{BFR} causes greater stress on the vasculature than LL and HL exercise, and that the leg press was generally more demanding than the leg extension. While additional cardiovascular measures were similar between LL_{BFR} and HL conditions, caution should be advised when prescribing BFR exercise for individuals with compromised cardiac or vascular function. Nevertheless, LL_{BFR} and HL exercise were perceived similarly, indicating that BFR training may be viable for healthy older people.

Keywords: strength training, skeletal muscle, blood pressure, cardiovascular, rating of perceived exertion

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INTRODUCTION

Sarcopenia is associated with increased risk of osteoporosis, cardiovascular complications, and decreases in functional independence and impaired performance in tasks of daily living (Marcell, 2003). These factors can result in a sedentary lifestyle, further exacerbating functional declines and increasing the likelihood of falls (Benichou and Lord, 2016). As such, techniques focused on delaying, stopping or reversing the age-related loss of muscle mass should be emphasized for clinicians working with these populations. Guidelines for older adults state that increasing muscle size and strength requires training with weights of ≥60% 1-repetition maximum (1RM), with many individuals requiring a gradual progressive overload to reach these weights (Ratamess et al., 2009). However, such progressions could take several months, increasing the likelihood of program attrition. Furthermore, musculoskeletal conditions that are common in an aging population (e.g., osteoarthritis, gout, back pain) can compromise strength and/or joint stability, resulting in an inability to safely perform exercise with heavy weights (Cook et al., 2017). Low-load resistance exercise with blood flow restriction (BFR), which utilizes inflatable cuffs around the top of the exercising limbs to occlude venous return but maintain arterial inflow (Scott et al., 2015a), represents an alternative training modality for an aging population.

Resistance training with BFR has been shown to enhance muscular size and strength for older adults in several studies (Karabulut et al., 2010; Vechin et al., 2015; Yasuda et al., 2016; Cook et al., 2017). These adaptations are observed when using BFR while lifting light weights (20–40% 1RM) that impose less mechanical stress than traditional training, and would otherwise not normally cause muscular development (Karabulut et al., 2010). Such findings have led researchers to advocate for BFR training to attenuate age-related declines in muscle mass and strength (Scott et al., 2015a). Irrespective of the potentially beneficial functional outcomes (Cook et al., 2017), the use of this technique by clinicians is still somewhat limited due to safety concerns (Spranger et al., 2015). Adding BFR during low-load resistance exercise in older adults can cause acute increases in systolic (SBP) and diastolic (DBP) blood pressure (Vieira et al., 2013; Staunton et al., 2015; Pinto and Polito, 2016), heart rate (HR) (Vieira et al., 2013; Staunton et al., 2015; Pinto and Polito, 2016), systemic vascular resistance (Pinto and Polito, 2016), and rate-pressure product (RPP) (Vieira et al., 2013; Staunton et al., 2015). These responses indicate an additional stress on the vasculature and amplified myocardial workload, prompting the need for caution when prescribing BFR training for older adults with hypertension, vascular dysfunction or heart disease.

The findings of possibly increased stress to the vasculature and myocardium during BFR are important; however, the current data may not accurately reflect normal resistance training in an aged population. No research has described the hemodynamic responses to different types of resistance exercise as they would be typically prescribed in a training session for older adults. The 45° leg press exercise (where the legs are raised above the level of the heart) is a common inclusion in training programs for older adults; yet, the incline position may alter the hemodynamics of this exercise when compared with more upright exercise such as the seated leg extension (where the legs are positioned below the level of the heart), despite both exercises primarily targeting the quadriceps femoris. Indeed, Staunton et al. (2015) have proposed that elevation of the legs during the 45° leg press may assist venous return, which could potentially mitigate increases in systemic vascular resistance in comparison with other exercises. The leg press also involves contribution from the hip extensor muscles, whereas the leg extension does not, which would likely increase the hemodynamic demands of exercise. In addition, few studies have examined the hemodynamic responses to BFR resistance exercise in older women. To our knowledge, the only published research in this population has recruited hypertensive patients (Pinto and Polito, 2016; Pinto et al., 2018), who may be contraindicated for BFR exercise (Kacin et al., 2015) and would therefore be unlikely to engage in this training stimulus. Finally, it is also important to consider how demanding participants perceive this training to be in comparison with more traditional heavy weights training. Adherence to exercise regimes has been identified as a major barrier for those prescribing training to older people (Room et al., 2017), and compliance is likely to decline if exercise is perceived as too difficult.

If BFR training is to become a more widely utilized training modality for older adults, it is imperative to understand the physiological impacts and potential for adverse events which may result from this exercise, as well as how this exercise is perceived by participants. Furthermore, it is also imperative to determine how low-load BFR exercise compares with more traditional heavy training. Should low-load BFR training become a viable alternative to lifting heavy weights for older people, comparisons between these two training structures are important to produce ecologically valid conclusions that can inform practice. The aims of this study were to determine the impacts of BFR on hemodynamic and perceptual responses during two different low-load resistance exercises within a training session, and compare these responses to traditional higher-load exercise.

MATERIALS AND METHODS

Participants

Fifteen older women (aged 63–75 years; **Table 1**) volunteered to participate in this study. Prior to commencement of the study, all participants were provided with information detailing the purpose and requirements of the research, and were screened for medical contraindications using a modified questionnaire designed specifically for BFR exercise (Kacin et al., 2015). All participants were non-smokers, had not undertaken structured resistance training within the previous 6 months, were not taking hormone replacement therapy, and did not present with any musculoskeletal, neurological, or vascular disease/injury. Participants were excluded if they presented with diabetes mellitus, hypertension, a history of blood clotting, or lymphedema. The study and its methods were approved by the Murdoch University Human Ethics Committee, and conducted

TABLE 1 | Characteristics of participants at baseline.

Variable	Mean ± SD	Range
Age (year)	66.8 ± 3.8	63–75
Body mass (kg)	65.8 ± 14.6	52.6–106.6
Height (m)	1.64 ± 0.06	1.53–1.75
BMI (kg m^2)	24.2 ± 4.4	18.8–34.8
SBP (mmHg)	120.2 ± 13.5	99–145
DBP (mmHg)	69.3 ± 7.4	57–89
MAP (mmHg)	86.3 ± 8.8	73.3–108.0
PP (mmHg)	51.0 ± 9.2	35–64
Leg Press 1RM (kg)	85.5 ± 26.8	52.5–150.0
Leg Extension 1RM (kg)	29.8 ± 5.8	21.3–38.8

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; 1RM, 1-repetition maximum. Values are presented as mean ± SD.

in accordance with the Declaration of Helsinki. All participants gave written informed consent prior to commencing the study.

Experimental Design

Participants reported to the laboratory at the same time of day on four occasions, each separated by 4–10 days. During their first visit, participants were familiarized with the rating of perceived exertion (RPE) and visual analog scales used in the research. They were then instructed on appropriate technique for the 45° leg press and the leg extension exercises, before performing 1RM testing following protocols described previously (Scott et al., 2015b). Briefly, participants 1RM were defined as their heaviest completed repetition, and was determined within 3–6 attempts. Using a crossover design, participants then visited the laboratory on three additional occasions to complete exercise trials in a randomized order: (1) low-load resistance exercise (LL), (2) low-load resistance exercise with BFR (LL_{BFR}), and (3) high-load resistance exercise (HL). During exercise trials, participants were monitored for hemodynamic function via blood pressure and automated impedance cardiography assessments. Participants also provided perceptual responses during and at 24 h following trials, to indicate perceived exertion and muscle soreness.

Exercise Trials

Upon arriving at the laboratory for exercise trials, participants rested quietly in a recumbent position for 15 min. Participants then warmed-up with 5 min of cycling at 60 RPM at a self-selected resistance, before commencing their assigned exercise protocol. During low-load trials, participants performed three sets of leg press and leg extension exercises with 20% 1RM, including 1 set of 20 repetitions followed by 2 sets of 15 repetitions, with 30 s recovery between sets and 8 min between exercises. During the high-load trial, participants performed 3 sets of 10 repetitions with 70% 1RM for each exercise, resting for 60 s between sets and 8 min between exercises. The low-load and high-load protocols were deliberately not matched for volume load (sets × repetitions × weight), in order to provide ecologically valid comparisons between how these strategies would actually be implemented in a training program (Bird et al., 2005). Similarly, the order of exercises was not randomized to reflect how these two exercises would be prescribed within a single training session; multi-joint exercises recruiting more muscle mass are recommended to be performed before single-joint exercises which utilize less muscle mass (Bird et al., 2005).

Determination and Implementation of BFR Pressure

Prior to LL_{BFR} trials, arterial occlusion pressure was measured to the nearest 10 mmHg with participants lying in a recumbent position using a handheld bi-directional ultrasound Doppler probe placed on the posterior tibial artery (MD6 Doppler, Hokanson, Bellevue, WA, United States) in accordance with established methods in BFR research (Loenneke et al., 2012). Restriction was applied to the proximal portion of the right thigh using a pressurized cuff (10 cm wide) connected to an E20 rapid cuff inflator and AG101 air source (Hokanson, Bellevue, WA, United States). During LL_{BFR} exercise, restrictive pressure was set to 50% of each participant's individualized arterial occlusion pressure. The cuffs were inflated for the duration of each exercise (including during inter-set rest periods), but were deflated between exercises.

Hemodynamic Responses

Measurements of HR, cardiac output (CO), and stroke volume (SV) were obtained during exercise at a beat-by-beat frequency and reported as 10 s mean values using automated impedance cardiography (Q-Link PhysioFlow PF-07, Manatec Biomedical, France). This non-invasive technology involves the application of electrodes to the neck and thorax (as per the manufacturer's instructions), from which the *trans*-thoracic bio impedance across the cardiac cycle and the electrical activity of the heart (i.e., electrocardiography) can be measured, to quantify hemodynamic variables. This method has been shown to be valid and reliable at rest and during submaximal exercise in patients with normal cardiorespiratory function (Charloux et al., 2000). Manual recordings of SBP and DBP were taken using a standard upper arm cuff and stethoscope (ALP K2, Tanaka Sangyo Co., Ltd., Japan; 12 cm wide internal bladder), at 1 min prior to commencing leg press and leg extension protocols, as well as immediately following the conclusion of each set of exercise. For all three conditions, these blood pressure measurements were obtained from the right arm, using the same equipment, and with the participant resting on the exercise equipment (for the leg press, participants' feet were resting on the floor rather than being pressed up against the machine platform). For the LL_{BFR} condition, the BFR cuffs were not inflated during the baseline measurement, but were inflated around participants' thighs during the post-set assessments. The use of manual recording of SBP and DBP has demonstrated smallest detectable differences of 7.6 and 7.0 mmHg, respectively, during rest conditions (Keavney et al., 2000). These data were used to calculate mean arterial pressure [MAP; calculated as 1/3 (SBP–DBP) + DBP] and pulse pressure (PP; calculated as

SBP–DBP). To provide details relevant to the highest potential cardiovascular demands during each set, the peak HR, CO and blood pressure measurements recorded during leg presses and leg extensions were used to calculate the highest potential RPP and total peripheral resistance (TPR) for each experimental session.

Perceptual Responses

A Category Ratio-10 RPE score was obtained immediately following each set, and a session RPE score was collected at 20 min following each trial to indicate the perceived difficulty of each set and the entire session, respectively. Participants also rated their muscle soreness at 24 h following each trial, by marking a 100 mm visual analog scale at a point between 0 (no soreness) and 100 (maximum soreness) (Kon et al., 2012). To anchor soreness to the lower body muscles, participants were instructed to flex and extend their knees before providing their soreness score.

Statistical Analyses

All data are represented as mean ± SEM unless indicated otherwise. Prior to analyses, the Shapiro–Wilk test confirmed that data were normally distributed. Following this, dependent variables during exercise (hemodynamic parameters and set RPE values) were compared between experimental trials (LL, LL_{BFR}, and HL) and time points (i.e., between sets within an exercise, and between matched sets in the leg press and leg extension) using linear mixed models. Trials and time points were set as fixed factors, and participants were set as random factors. Where a significant main effect or interaction was observed, Fisher's LSD *post hoc* assessment was used to identify where differences occurred. Session-RPE and muscle soreness scores were also analyzed via linear mixed models, with trials set as fixed factors and participants as random factors, and Fisher's LSD *post hoc* implemented where a significant main effect was observed. The gradient of the increase in set RPE scores was calculated as $\Delta Y \div \Delta X$; the change in RPE from set 1 to 3 divided by the change in set number (i.e., 3−1 = 2). These gradient values were calculated for all participants during each trial, and then compared between conditions and exercises via linear mixed models as described above. Effect sizes were calculated to determine the magnitude of differences between all comparisons made for trials and time points as Cohen's d_z (difference in the mean divided by the standard deviation of the difference; 0.20–0.49 = small effect; 0.50–0.79 = moderate effect; and ≥0.80 = large effect) (Cohen, 1988). Statistical analyses were conducted using SPSS (v24, Chicago, IL, United States), with statistical significance set at $p \leq 0.05$.

RESULTS

A high level of adherence was observed in this study with only one participant failing to complete all required sets. In this one individual, only the third set of leg press in the LL_{BFR} condition was not completed. Discussion with this participant revealed that they stopped the set due to a zip on their pants being compressed against their leg by the BFR cuffs, which caused localized pain. The participant was able to re-position the cuffs so that they no longer compressed the zip, and then completed all sets of the leg extension exercise without any further pain.

An interaction was observed for volume load between exercise and protocol ($p < 0.001$), with *post hoc* analyses confirming higher volume load scores for both exercises during the high-load protocol (total volume load = 2421 ± 171 kg) compared with the low-load (total volume load = 1153 ± 82 kg) trials ($p < 0.001$; d_z = 3.19–5.16), as well as higher volume loads for the leg press compared with the leg extension exercise for the high-load (leg press = 1796 ± 146 kg, leg extension = 626 ± 31 kg; $p < 0.001$; d_z = 2.47) and low-load (leg press = 855 ± 69 kg, leg extension = 298 ± 15 kg; $p < 0.001$; d_z = 2.47) protocols.

Hemodynamic Responses

The blood pressure responses for each trial are shown in **Figure 1**. For SBP, DBP, and MAP, significant interactions between time and trial were observed ($p \leq 0.02$). *Post hoc* analyses determined that SBP was increased from baseline following each set for leg press and leg extension exercise during LL ($p \leq 0.002$; d_z = 1.54–2.66), LL_{BFR} ($p < 0.001$; d_z = 1.60–3.04), and HL ($p < 0.001$; d_z = 1.52–2.20) trials. SBP was also higher after all sets in the leg press compared to matched sets in the leg extension for all conditions ($p \leq 0.001$; d_z = 1.10–2.14). Furthermore, LL_{BFR} trials produced higher SBP values after each set compared with both LL ($p \leq 0.021$; d_z = 1.30–2.15) and HL ($p \leq 0.016$; d_z = 0.97–1.86) sessions. DBP was increased from baseline only during the LL_{BFR} trial, for each set for leg presses and sets 2–3 for leg extensions ($p \leq 0.001$; d_z = 1.49–2.77), and was higher after sets 2 and 3 in the leg press compared to matched sets in the leg extension only during LL_{BFR} ($p \leq 0.028$; d_z = 1.38–1.53). Higher DBP values were also observed during LL_{BFR} compared with LL ($p \leq 0.001$; d_z = 1.16–2.04) and HL ($p \leq 0.004$; d_z = 1.21–1.82) for all sets. The MAP responses were similar to SBP, with increases from baseline observed following each set during LL ($p \leq 0.030$; d_z = 1.35–3.15), LL_{BFR} ($p < 0.001$; d_z = 1.52–3.63), and HL ($p < 0.037$; d_z = 0.96–1.94) trials, and greater values after all sets in the leg press compared to matched sets of the leg extension for all conditions ($p \leq 0.031$; d_z = 1.24–1.85). Higher values were recorded during LL_{BFR} compared with LL ($p < 0.001$; d_z = 1.17–2.60) and HL ($p \leq 0.001$; d_z = 1.21–2.07) trials. Furthermore, SBP, DBP, MAP values were not different between HL and LL trials at any point. Regarding PP, a significant main effect was only observed for time ($p < 0.001$), with *post hoc* analyses indicating increases from baseline during exercise were observed for all three trials ($p < 0.001$; d_z = 1.37–2.53), as well as greater values after all sets in the leg press compared to matched sets of the leg extension ($p \leq 0.001$; d_z = 0.95–1.68).

Additional cardiovascular responses to each exercise trial are presented in **Table 2**. To reflect the periods of greatest cardiovascular demand during exercise, these factors are reported as the peak values calculated during leg press and leg extension exercises. For HR responses, significant main

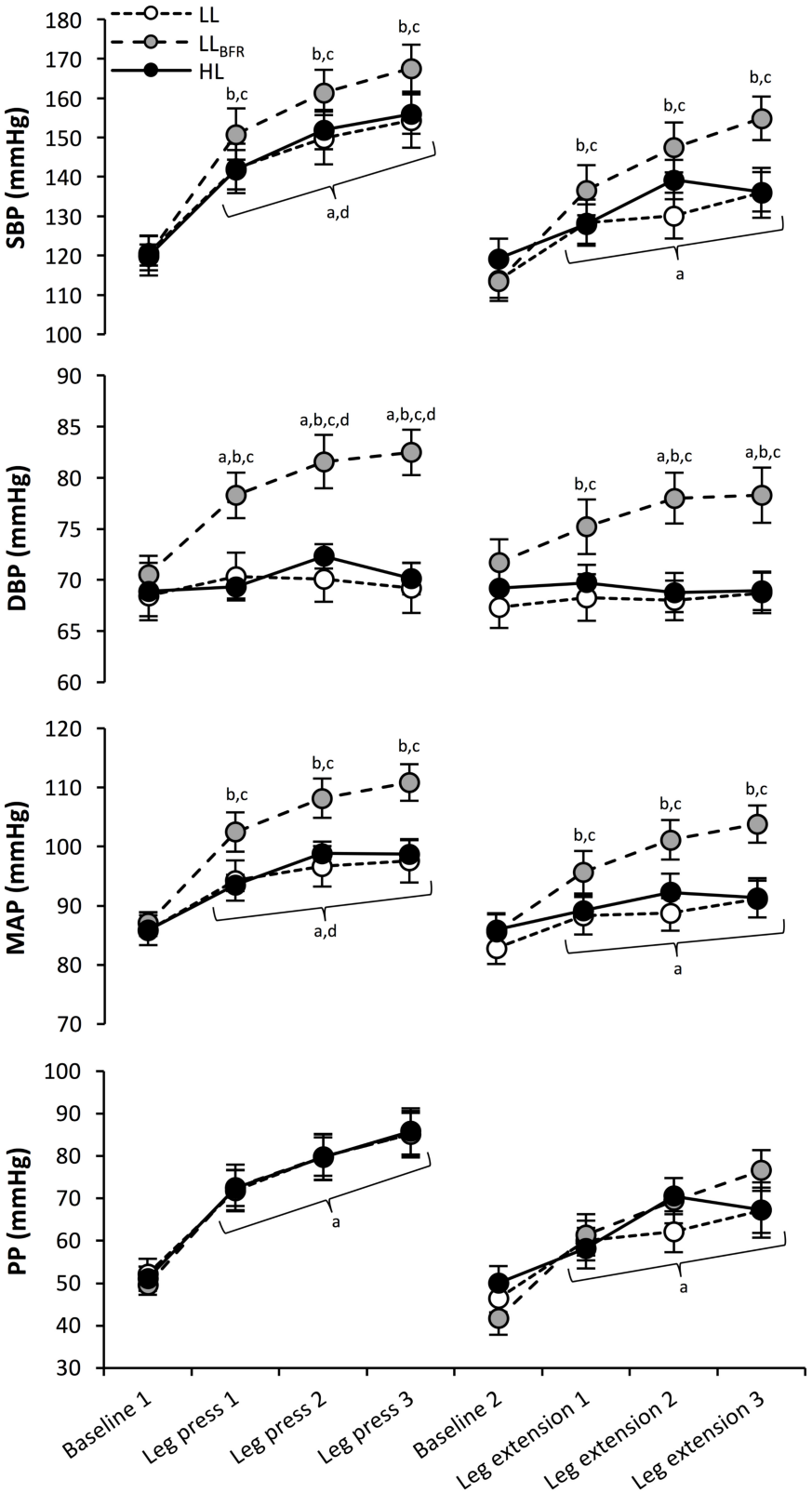

FIGURE 1 | Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP) responses at baseline for each exercise, and immediately following each set (brackets denote differences for all three conditions). [a]Significantly different to pre-exercise baseline. [b]Significantly different to HL at the same time point. [c]Significantly different to LL at the same time point. [d]Significantly different to leg extension exercise for matched set.

TABLE 2 | Cardiovascular responses to exercise trials.

	Low-load	Low-load with BFR	High-load
Leg press			
Heart rate (bpm)	108.9 ± 4.6[a]	115.5 ± 6.0[a,b]	118.7 ± 3.8[a,b]
Cardiac output (l·min^{-1})	11.7 ± 0.9[a]	13.9 ± 1.9[a]	13.8 ± 1.2[a]
Stroke volume (ml)	125.6 ± 8.4[a]	138.4 ± 14.2[a]	135.0 ± 10.4[a]
Rate-pressure product	171.2 ± 12.4[a]	192.3 ± 16.9[a,b]	181.7 ± 7.7[a,b]
Total peripheral resistance	9.0 ± 0.6[a]	8.9 ± 0.9[a]	7.6 ± 0.6[a]
Leg extension			
Heart rate (bpm)	101.4 ± 2.3	111.9 ± 4.2[b]	109.1 ± 3.2[b]
Cardiac output (l·min^{-1})	10.0 ± 0.7	10.4 ± 0.9	10.3 ± 0.5
Stroke volume (ml)	106.8 ± 6.6	113.6 ± 15.2	104.6 ± 5.2
Rate-pressure product	139.5 ± 7.4	167.2 ± 9.0[b]	156.0 ± 9.3[b]
Total peripheral resistance	9.8 ± 0.8	10.6 ± 0.9	9.4 ± 0.4

[a]*Significantly different to leg extension within same condition.* [b]*Significantly different to low-load.*

effects were observed for trial and exercise ($p \leq 0.001$), but not for the interaction between variables. *Post hoc* analyses indicated significantly higher HR during leg press compared with leg extensions across all trials ($p < 0.001$; d_z = 0.83–1.35), and that the LL protocol resulted in significantly lower HR compared with LL_{BFR} ($p = 0.002$; d_z = 1.22–1.51) and HL ($p = 0.001$; d_z = 1.51–1.58) trials. Significant main effects for exercise were observed for CO ($p < 0.001$), with *post hoc* analyses indicating significantly higher CO during leg press compared with leg extensions ($p < 0.001$; d_z = 0.79–1.23). Significant main effects were also observed for exercise in SV ($p < 0.001$). *Post hoc* analyses indicated significantly higher SV during leg press compared with leg extensions ($p = 0.002$; d_z = 0.84–1.21). Regarding RPP calculations, significant main effects were observed for trial and exercise ($p < 0.001$), but not for the interaction between variables. *Post hoc* analyses indicated significantly higher RPP during leg press compared with leg extensions across all trials ($p < 0.001$; d_z = 1.07–1.48). Furthermore, the LL protocol produced significantly lower RPP values compared with LL_{BFR} ($p \leq 0.001$; d_z = 1.65–2.54) and HL ($p = 0.011$; d_z = 1.16–1.29) trials. For TPR responses, a significant main effect was only noted for exercise ($p = 0.015$), with *post hoc* analyses confirming higher values during the leg extension compared to the leg press ($p = 0.015$; d_z = 1.07–1.36).

Perceptual Responses

The RPE values for each set of exercise and session RPE values for each trial are presented in **Figure 2**. An interaction between trial and time were observed for the RPE of each set during experimental trials ($p \leq 0.031$). *Post hoc* analyses confirmed that RPE tended to increase across sets for both exercises in each trial ($p \leq 0.035$; d_z = 0.84–1.68). The set RPE scores were lower for every set during LL compared with LL_{BFR} ($p \leq 0.008$; d_z = 1.17–1.59) and HL ($p < 0.001$; d_z = 0.97–1.76), while set 1 of the leg press was lower in LL_{BFR} compared with HL ($p = 0.008$; d_z = 0.87). Regarding the gradient of increase in set RPE scores, significant main effects were observed for trial and exercise ($p \leq 0.001$), but not for the interaction between variables. *Post hoc* analyses indicated a significantly larger gradient (i.e., rate of increase in RPE) during LL_{BFR} compared to LL ($p < 0.001$; d_z = 1.04–1.06) and HL ($p < 0.001$; d_z = 1.08–1.17). Furthermore, RPE increased faster for the leg press compared with the leg extension ($p < 0.001$; d_z = 1.01–2.84).

For session RPE, a main effect was observed for condition ($p < 0.001$), with LL resulting in lower scores compared with LL_{BFR} ($p < 0.001$; d_z = 1.68) and HL ($p < 0.001$; d_z = 0.93). There were no differences in session RPE between LL_{BFR} and HL. Perceived muscle soreness at 24 h post exercise was low for all conditions (HL = 10.8 ± 7.4 mm, LL_{BFR} = 9.4 ± 4.1 mm, and LL = 1.4 ± 0.7 mm), with no main effect for condition observed. Unfortunately, not all participants provided a session-RPE score following the LL_{BFR} (13 responses) or the HL (12 responses) trials, and similar problems were encountered in obtaining muscle soreness values at 24 h following the LL (14 responses), LL_{BFR} (14 responses), and HL (12 responses) trials.

DISCUSSION

The main findings of this study indicate that, (1) adding BFR to light-weight resistance exercise increases SBP, DBP, and MAP to values which exceed those observed during traditional higher-load exercise, (2) LL_{BFR} and HL exercise resulted in similar HR responses and myocardial workload, which were greater than during LL exercise, (3) LL_{BFR} and HL were perceived to be of similar difficulty, while LL was reported as less strenuous, and (4) the leg press was more demanding than the leg extension, and these effects may be exaggerated by BFR. These findings provide new insights regarding the safety of LL_{BFR} training for older women; while this population has been proposed to benefit from a BFR training approach (Cook et al., 2017), very few studies have examined cardiovascular responses in these individuals or the differences between different types resistance exercises when combined with BFR.

While it may be expected that higher-load exercise could exacerbate blood pressure responses (Williams et al., 2007), in the current study we observed similar SBP, DBP, and MAP responses in the LL and HL protocols. These findings are likely due to the different number of repetitions performed between light and heavy training sessions. For example, Polito et al. (2007) demonstrated similar blood pressure responses between heavy weights training with few repetitions compared with lighter weights training for more repetitions. Importantly, LL_{BFR} resulted in elevated blood pressures when compared with LL and HL, findings similar to those previously reported for older hypertensive (Pinto and Polito, 2016) and normotensive (Vieira et al., 2013; Sardeli et al., 2017) individuals, as well as for healthy young men (May et al., 2017). These findings have practical relevance for practitioners,

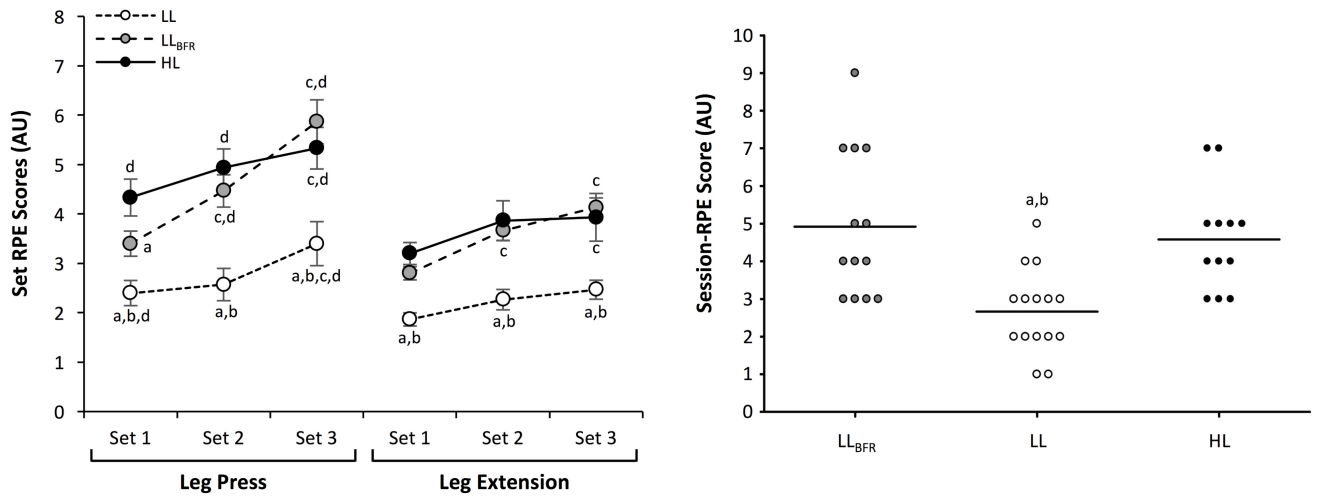

FIGURE 2 | Set and session-rating of perceived exertion (RPE) scores for low-load, low-load with blood flow restriction, and high-load exercise protocols. To provide information regarding individual response to exercise, session-RPE scores have been displayed as individual data points, with mean values indicated by a bold line. [a]Significantly different to HL. [b]Significantly different to LL$_{BFR}$. [c]Significantly different to Set 1 for same exercise. [d]Significantly different to leg extension exercise for matched set.

especially if they are considering the implementation of BFR training in hypertensive patients. Nevertheless, the observed increases in blood pressures were well below those previously associated with hemorrhagic events (Haykowsky et al., 1996), and consistent with commonly observed changes in blood pressure during graded exercise tests (Olson et al., 2012).

The HR responses in this study indicate that the addition of BFR to the LL exercise confers greater cardiovascular work for the same mechanical stimulus. These findings are consistent with previous reports investigating BFR exercise in older people (Vieira et al., 2013; Staunton et al., 2015; Pinto and Polito, 2016), and are likely the result of an elevated chemoreflex (Victor and Seals, 1989) in response to the well-documented increases in metabolic stress during BFR exercise (Suga et al., 2012; Vieira et al., 2013; Spranger et al., 2015). Interestingly, the elevated HR during LL$_{BFR}$ was comparable to that observed during HL. Similar results have been observed by Pinto et al. (2018), who reported comparable responses to resistance training with (3 × 10 repetitions with 20% 1RM) and without (3 × 10 repetitions with 65% 1RM) BFR. However, the same research group have also reported that using BFR during 3 × 15 repetitions with 20% 1RM causes elevated HR responses compared with 3 × 8 repetitions with 65% 1RM (Pinto and Polito, 2016). These contrasting findings indicate that HR responses to light and heavy resistance exercise can be influenced by BFR and the repetition volume of each set. As such, it is important to consider that the findings from the current study are relevant to exercise performed for a pre-determined number of repetitions in each set, and not to volitional fatigue. Future research should therefore aim to assess how manipulation of acute exercise variables (e.g., load lifted, number of sets and repetitions, lifting tempo) impacts on the physiological responses to exercise with BFR.

In this study, the heavy and light weight protocols were deliberately not matched for volume load in order to quantify the hemodynamic implications when performing these exercises in a typical manner for increasing muscle mass and strength (Scott et al., 2015a). Despite the differences in mechanical demands, LL$_{BFR}$ and HL training were perceived to be of similar difficulty, and both were rated as more strenuous than LL. An interesting observation during exercise was that RPE scores for each set increased more quickly across the protocol in LL$_{BFR}$ compared with HL and LL. It is possible that the more rapid increase in perceived intensity during LL$_{BFR}$ is related to the increase in metabolite accumulation, which is known to occur with each set in BFR exercise (Suga et al., 2012). Particularly when BFR is maintained during the inter-set rest periods in the absence of muscular contractions, as was performed in the current study, the venous occlusion caused by cuffs will limit the removal of metabolites from the limb and compound the local metabolite buildup. However, some authors have suggested that perceived exertion is independent of afferent feedback from skeletal muscle during exercise (Marcora, 2009). Similar perceptual responses to those observed in the current study have been previously reported, whereby a progressive increase in RPE was observed during knee extension exercise in unrestricted high-load (4 × 10 with 70% 1RM) and low-load BFR (1 × 30 and 3 × 15 with 20% 1RM, BFR set at 60% arterial occlusion pressure) conditions (Loenneke et al., 2015). Furthermore, a trend for more rapid increases in RPE during low-load BFR exercise (45% increase from set 1 to 4), compared to during high-load exercise (25% increase from set 1 to 4) was observed (Loenneke et al., 2015). Collectively, these findings indicate that low-load BFR exercise is perceived as similar in difficulty to high-load exercise if ≤4 sets are performed, but could potentially be perceived as more difficult if a substantially greater number of sets are undertaken.

Similar to set RPE scores, session RPE was lowest following the LL condition; yet, comparable between LL_{BFR} and HL. Furthermore, very low levels of muscle soreness with no differences between protocols were observed at 24 h after exercise for all three exercise methods. These data indicate that older women tolerate LL_{BFR} training similarly to more traditionally prescribed higher-load exercise. Considering that perceived difficulty is often cited as a barrier to undertaking resistance training for older adults (Fisher et al., 2017), it appears that adherence to a LL_{BFR} training program may be similar to a high-load unrestricted training program. It must be acknowledged, however, that there are currently no long-term studies which have examined the adherence rates of LL_{BFR} training programs compared with more traditional exercise prescription. If BFR training is to become a viable training strategy for attenuating sarcopenia in older adults, this gap in scientific understanding should be addressed.

Considering the comparison between exercises (i.e., leg extension vs. leg press), higher SBP, MAP, and PP were observed for the leg press compared with matched sets of leg extensions for all conditions. Greater peak HR, CO, SV, and RPP were also observed during the leg press compared with the leg extension. An interesting finding from our study was that DBP was increased in the leg press only during sets 2 and 3 of the LL_{BFR} condition. While this may indicate an exercise-specific response to BFR (i.e., the effects are more pronounced in the leg press than the leg extension), mean values for DBP were <83 mmHg (**Figure 1**), and the magnitude of these changes may not be clinically important. The difference between exercises may be due to the larger muscle mass required to complete the leg press compared with the leg extension, and the position of the participants during the 45° leg press. For instance, during the leg press the active muscle mass is elevated above the heart, necessitating greater force to increase blood flow against the influence of gravity. Furthermore, gravity-enhanced venous return would result in greater central blood volume, and therefore increased SV due to the Frank–Starling mechanism. Importantly, both LL_{BFR} and HL demonstrated greater cardiovascular workload (RPP) compared with LL (main effects). Analyses of the rate of change in RPE across sets also indicated that the leg press had a larger gradient of increase in perceived exertion compared with the leg extension (**Figure 2**). This provides evidence that not only was the leg press more physiologically challenging compared to the leg extension, but it was perceived to become more difficult with each set than the leg extension. Taken together, these collective findings indicate that both the addition of BFR and choice of resistance exercise may influence cardiovascular function during resistance training and therefore need to be considered during prescription.

While this study has provided novel insights regarding the hemodynamic responses to different exercises performed with BFR, some limitations with the experiment should be acknowledged. Firstly, while the level of BFR during exercise was individualized to each participant's resting arterial occlusion pressure, it is possible that the dissimilar postures during the leg press and leg extension caused different changes in blood flow for the same given BFR pressure. An alternative to this would be to measure arterial occlusion pressure in the specific posture required for each individual exercise prior to training. Nevertheless, considering the within-subject design of our experiment, the between condition comparisons reported in this paper would not have been affected by this limitation. Secondly, the manual assessment of blood pressure during this study was limited to the immediately post-set time point and the impedance cardiography data was collected as 10 s mean values. It is possible that the blood pressure responses during each set (i.e., prior to our measurement time point) were different to the responses we measured, or that the 10 s window of each impedance cardiography data point may have blunted the hemodynamic variables assessed. In light of this, we have reported the peak values for several hemodynamic variables in this study to provide an indication of the highest measured physiological demands during each exercise. Nevertheless, future research should aim to investigate hemodynamic responses to BFR exercise in healthy older women further via continuous blood pressure monitoring technologies. Finally, it should be acknowledged that not all participants provided perceptual responses to the research team after the conclusion of their trials. This was taken into account with the analyses conducted, as linear mixed models are better able to accommodate missing data points than common analysis of variance methods.

CONCLUSION

The findings from this study indicate that LL_{BFR} caused greater blood pressures than more traditional heavy training, despite much lower mechanical demands and other cardiovascular and perceptual responses being similar between LL_{BFR} and HL conditions. Interestingly, the leg press exercise generally conferred greater cardiovascular and perceptual responses than the leg extension, which may even be exaggerated by the application of BFR (particularly for DBP). While BFR training with light weights could be beneficial for older people to attenuate sarcopenia without heavy resistance training, our results suggest that caution should be taken if implementing BFR for individuals with hypertension or reduced myocardial ischemic thresholds. For these individuals, smaller muscle mass exercises such as leg extensions may not increase hemodynamic stress as much as a leg press, though the practitioner should also be aware that a single-joint exercise may have more limited transfer to multi-joint tasks of daily living.

AUTHOR CONTRIBUTIONS

BS, JP, and KH developed the concept for this research project. HT performed all recruitment and screening. HT and KM performed all data collection. BS and KM performed data and statistical analyses. BS drafted the original manuscript. All authors made significant contributions to editing and finalizing the manuscript and also approved the final version of the manuscript.

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Muscle Adaptations to High-Load Training and Very Low-Load Training With and Without Blood Flow Restriction

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An inability to lift loads great enough to disrupt muscular blood flow may impair the ability to fatigue muscles, compromising the hypertrophic response. It is unknown what level of blood flow restriction (BFR) pressure, if any, is necessary to reach failure at very low-loads [i.e., 15% one-repetition maximum (1RM)]. The purpose of this study was to investigate muscular adaptations following resistance training with a very low-load alone (15/0), with moderate BFR (15/40), or with high BFR (15/80), and compare them to traditional high-load (70/0) resistance training. Using a within/between subject design, healthy young participants (n = 40) performed four sets of unilateral knee extension to failure (up to 90 repetitions/set), twice per week for 8 weeks. Data presented as mean change (95% CI). There was a condition by time interaction for 1RM ($p < 0.001$), which increased for 70/0 [3.15 (2.04,4.25) kg] only. A condition by time interaction ($p = 0.028$) revealed greater changes in endurance for 15/80 [6 (4,8) repetitions] compared to 15/0 [4 (2,6) repetitions] and 70/0 [4 (2,5) repetitions]. There was a main effect of time for isometric MVC [change = 10.51 (3.87,17.16) Nm, $p = 0.002$] and isokinetic MVC at 180°/s [change = 8.61 (5.54,11.68) Nm, $p < 0.001$], however there was no change in isokinetic MVC at 60°/s [2.45 (−1.84,6.74) Nm, $p = 0.261$]. Anterior and lateral muscle thickness was assessed at 30, 40, 50, and 60% of the upper leg. There was no condition by time interaction for muscle thickness sites (all $p \geq 0.313$). There was a main effect of time for all sites, with increases over time (all $p < 0.001$). With the exception of the 30% lateral site ($p = 0.059$) there was also a main effect of condition (all $p < 0.001$). Generally, 70/0 was greater. Average weekly volume increased for all conditions across the 8 weeks, and was greatest for 70/0 followed by 15/0, 15/40, then 15/80. With the exception of 1RM, changes in strength and muscle size were similar regardless of load or restriction. The workload required to elicit these changes lowered with increased BFR pressure. These findings may be pertinent to rehabilitative settings, future research, and program design.

Keywords: ischemia, resistance training, volitional failure, kaatsu, exercise

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INTRODUCTION

Studies have shown that training with lower loads (i.e., 30% 1RM) to volitional failure elicits increases in muscle size and strength similar to high-load resistance training (Mitchell et al., 2012; Morton et al., 2016). However, there may be a point at which the external training load is too low to fully stimulate muscular adaptations. A 12-week training protocol comparing 70% 1RM and 15.5% 1RM, found muscle size and strength adaptations favored the high-load condition (Holm et al., 2008). The results, however, may be limited by the methodology of matching exercise volume (i.e., total kg lifted; reps × load) in the 15.5% 1RM to that completed during 70% 1RM. While exercise to failure is not always necessary for adaptation, it has been suggested as a more appropriate strategy to truly compare the efficacy of exercise protocols (Dankel et al., 2016).

During dynamic exercise, the ability to reach volitional failure at the muscular level may depend on generating a contraction strong enough to disrupt muscle blood flow. At very-low isometric contraction intensities the changes in mean arterial pressure are small and may lead to a prolonged contraction time (Hunter and Enoka, 2001). If the exercise load is too low and does not induce some level of fatigue the muscular size and strength adaptation may be attenuated, or may be more aerobic in nature, evidenced by a greater acute mitochondrial protein synthetic response vs. myofibrillar (Burd et al., 2012). As Holm et al. (2008) did not train to failure, it is currently unknown if doing so with a very low-load can stimulate similar muscular adaptations when compared to a traditional high-load protocol.

Applying blood flow restriction (BFR) to the limb is a strategy used to disrupt muscular blood flow and increase fatigability of the muscle during low-load exercise (Ganesan et al., 2015). Over a training program, the addition of BFR significantly reduced the workload required to reach volitional failure, while eliciting similar muscular adaptations compared to non-restricted training at the same relative load (Fahs et al., 2015; Farup et al., 2015). However, the minimum level of pressure necessary for maximal adaptation is unclear: using 30% 1RM Counts et al. (2016) found no difference in muscle hypertrophy between training with high [i.e., 90% arterial occlusion pressure (AOP)] or moderate pressures (40% AOP), whereas Lixandrao et al. (2015) found a greater hypertrophic effect when using a high pressure (i.e., 80% AOP) in conjunction with 20% 1RM, albeit when not exercising to failure. Thus, suggesting a higher pressure (80% AOP) may be necessary when using loads lower than 30% 1RM.

The purpose of the current study was to compare muscle size, strength, and endurance adaptations between high-load and very-low load training to volitional failure. In addition, we sought to determine if applying BFR was necessary to induce adaptation to very low-load training, and if the effect of BFR was pressure dependent. As very low-load exercise may be necessary and/or preferable for certain populations, the findings of this study would give more insight into program design, providing more understanding of the required stimuli (i.e., loading thresholds and restriction application) for muscle size and strength increases. We hypothesized that: both strength and muscle size increases would be augmented in a pressure dependent manner within the 15% 1RM conditions, strength increases would be greatest with 70% 1RM, the 80% BFR condition would be needed to increase muscle size similar to 70% 1RM, and endurance would be greater in all 15% 1RM conditions compared to 70% 1RM.

MATERIALS AND METHODS

Participants

Forty-six participants, between the ages of 18–35 years, were recruited to participate in the study. Participants were untrained and had not engaged in resistance exercise within 6 months prior to beginning the study. Participants were excluded from the study if: they regularly used tobacco products within the previous 6 months, had a BMI $\geq$ 30 kg/m^2, an orthopedic injury preventing exercise, or took medication for hypertension. Although it has been shown to be relatively safe, some concern regarding the risk of thromboembolism exists regarding BFR exercise. Thus, participants were also excluded if they met at least two of the following risk factors for thromboembolism: diagnosed with Crohn's disease, past fracture of the hip, pelvis, or femur, major surgery within the last 6 months, varicose veins, family or personal history of deep vein thrombosis, family or personal history of pulmonary embolism (Motykie et al., 2000). Four participants dropped out of the study prior to beginning training, while two others dropped out during the training period due to personal reasons. No adverse responses to training were observed or reported. The data were analyzed and presented for the 20 males and 20 females completing all visits (with the exception of one individual who missed one training session). This study was approved by The University of Mississippi's Institutional Review Board. All participants gave written informed consent in accordance with the Declaration of Helsinki.

Experimental Design

Over the course of 22 total visits, spanning 10 weeks, muscle size, strength, and endurance of the knee extensors were measured before and after an 8-week unilateral knee extension training protocol. Participants trained with two of four possible conditions, one assigned to each leg. The conditions, labeled as % 1RM/% AOP, were: 70/0, 15/0, 15/40, and 15/80. They were assigned in a randomized, counter-balanced fashion, with no participant receiving the same condition in both legs. On the initial pre-visit, if the participant met inclusion criteria, they proceeded to sign a written informed consent document and PAR-Q, then had height and body mass assessed, followed by muscle thickness measurements of both legs. On the second pre-visit, participants were familiarized with procedures to be used for testing knee extension 1RM as well as isometric and isokinetic strength. On the third pre-visit, participants completed knee extension 1RM tests for each leg, then completed isokinetic and isometric strength tests followed by muscular endurance assessment. On visits 4–19 participants completed the 8-week training protocol, training twice per week with a minimum of 24 h separating each visit. Post measurements were taken

over three separate days, at least 48 h following the last training session, and resembled pre-training test procedures. Additional measures included a mid-point assessment of muscle thickness, and an assessment of the acute exercise-induced swelling response during training sessions 1, 9, and 15. Of note: the current experiment presented herein was part of a larger training program that also included an upper body training protocol (upper body data reported elsewhere).

Muscle Thickness

Muscle thickness was measured using B-mode ultrasound (Logiq-e GE, Fairfield, CT, United States) before training, prior to training session 9 (mid-training), and 48 + h after the last training session. While the participant was standing, feet shoulder width apart and weight evenly balanced, a linear array probe (L4-12t GE, Fairfield, CT, United States) was coated with transmission gel and placed against the skin perpendicular to the femur, with care taken not to depress the dermal surface. Two images were saved and stored for each site on the anterior and lateral portion of both legs (30, 40, 50, and 60% of the distance from the greater trochanter to the lateral condyle of the femur). To include an internal measurement control, an additional image was taken on the participant's left posterior upper arm midway from the acromion process to the olecranon process. Muscle thickness was determined as the average distance between the muscle-bone and muscle-adipose interfaces, assessed to the nearest 0.01 cm, from the two stored images. All measurements and analyses of muscle thickness were taken by the same investigator throughout the study. To limit any bias, the investigator was blinded to each condition during image analysis, which was done only after all testing was completed.

One-Repetition Maximum

1RM was used as a strength outcome and to determine training loads. 1RM was assessed by finding the greatest load participants could lift one time, with proper form, through a full range of motion using a unilateral knee extension machine (Hammer Strength Iso-Lateral Leg Extension Life Fitness, Rosemont, IL, United States). Prior to testing, as a warmup, a self-determined number of unloaded repetitions were completed, followed by 1 repetition each of an estimated 30 and 70% 1RM. For testing, participants were asked to move a given load from a starting position (knee angle of approximately 90°) to full knee extension one time per attempt, while buckled into the seat with arms crossed over their chest. In an effort to reduce subjectivity, a bar was placed at the top of the range of motion and for the attempt to be classified as successful, the load had to reach the bar. The load was increased following each successful attempt. If unsuccessful, the load was decreased and this process continued until the maximum load the participant could successfully lift was determined. The amount of weight added or removed after each attempt was based upon the speed and effort from the previous attempt. Attempts for each leg were alternated and at least 45 s of rest was observed between attempts (90 s between attempts using the same leg). All testing was supervised by trained personnel.

Isometric and Isokinetic Strength

Isometric and isokinetic strength were tested using a dynamometer (Quickset System 4 Biodex, Shirley, NY, United States). Prior to all testing, chair and leg attachments were adjusted to properly fit each individual, then settings were recorded to ensure the same testing conditions for all measures. Isokinetic testing was performed at two speeds, 180°/s and 60°/s. While seated, participants performed 2 sets (separated by 60 s rest) of 3 maximal knee extensions (knee angle from approximately 90° to 180°) at 180°/s then at 60°/s. During isometric testing, participants completed two maximal knee extensions with the knee positioned at approximately 90° of flexion. Participants completed two attempts with 60 s rest. Attempts were given up to 15 s, but were stopped prior if a clear decrease or plateau in torque was observed. This resulted in most attempts lasting approximately 3 – 8 s. All testing was performed with participants' arms crossed over their chest. Participants were also provided with visual feedback and strong verbal encouragement during each attempt. Regarding test order, isokinetic testing was always completed prior to isometric and all three tests were completed on one leg first (randomized), followed by the contralateral leg.

Endurance

To compare changes in muscular endurance between conditions, participants were asked to complete one set of unilateral knee extension exercise to volitional failure before and after training. The load for pre and post endurance tests was 42.5% of the participants' pre-training 1RM value as this load was exactly halfway between 15 and 70% 1RM. This relative load was chosen to avoid favoring one loading condition over the other. Prior to testing the seat was adjusted and recorded so that all testing conditions were similar. A lap belt was pulled snuggly across participants' waist and they were instructed to maintain arms crossed over their chest while the test was being conducted. Participants were instructed to lift the load from the starting position until touching a bar set at the top of the range of motion for a repetition to be deemed successful. Repetitions were performed at a cadence of 2 s per contraction (1 s concentric and 1 s eccentric). If a participant was unable to complete a full range of motion or maintain proper cadence, the test was terminated. A 5 min rest period was observed between tests.

Arterial Occlusion Pressure

To apply a relative pressure during 15/40 and 15/80 conditions, AOP was taken prior to exercise while the participant was seated in a knee extension machine. A 10 cm wide nylon cuff (SC10 Hokanson, Bellevue, WA, United States) was applied to the proximal portion of the thigh. An auditory signal of a pulse was found at the posterior tibialis artery using a Doppler probe (MD6 Hokanson, Bellevue, WA, United States). Starting at 50 mmHg the cuff was slowly inflated (E20 Rapid Cuff Inflator Hokanson, Bellevue, WA, United States) until the pulse distal to the cuff was no longer detected. The inflation pressure of the cuff was recorded as AOP and the assigned percentage (40 or 80%) of this pressure was applied during exercise.

Training Protocol

The 8-week training protocol required 2 supervised training sessions per week, consisting of 4 sets of unilateral knee extensions to volitional failure under the assigned condition. Both legs trained each day with the leg training first alternated between days. Participants were given a self-determined rest period between training each leg. The very low-load conditions (15/0, 15/40, and 15/80) trained with a load equal to 15% of 1RM and had inter-set rest periods of 30 s. The high-load condition (70/0) trained with a load equal to 70% 1RM with 90 s inter-set rest periods. Applied pressure during the BFR conditions [15/40 (40% AOP) and 15/80 (80% AOP)] was set as a percentage of pre-exercise AOP measured each session while participants were in an upright seated position. A 10 cm wide inelastic cuff (SC10 Hokanson, Bellevue, WA, United States) was applied to the most proximal portion of the leg, inflated (E20 Rapid Cuff Inflator Hokanson, Bellevue, WA, United States) prior to exercise, and remained inflated until the cessation of the last set, after which it was deflated and removed. All repetitions were performed to a metronome (1 s concentric and 1 s eccentric). All sets, regardless of condition were ceased at 90 repetitions, as this would equal the time-frame used by Holm et al. (2008), and it would minimize participant strain. Further, if the contractions were not generating a sufficient amount of fatigue the stimulus would likely become more aerobic with time (Burd et al., 2012). To minimize any confounding effects of load on failure, loads were not progressed across training. To minimize soreness associated with novel exercise, sets were ramped at the beginning of training (i.e., training session one, participants completed one set of exercise, another set was added for session two, three sets were completed for sessions three and four, and thereafter four sets of exercise were completed for all remaining sessions).

Exercise-Induced Swelling

Measures of the acute exercise-induced swelling response were assessed at training sessions 1, 9, and 15 to better determine if chronic changes in muscle thickness were due to swelling rather than muscle growth as a muscle's ability to swell provides some indication that there is not a large presence of swelling at baseline (Buckner et al., 2017a). The anterior 50% site was measured immediately before and after the exercise protocol using procedures similar to those of muscle thickness except images were frozen and muscle thickness measured immediately using on-screen calipers. Two images were analyzed and an average of the two measures was determined to be muscle thickness. If the two initial measures differed by greater than 0.1 cm, a third image was taken and an average of the two closest measures was used.

Statistical Analyses

SPSS version 24.0 (SPSS Inc., Armonk, NY, United States) was used for data analysis. To examine changes in all strength, muscle thickness, and exercise volume values across time between groups, while accounting for our within/between subject design, two-factor (condition × time) analysis of variance was used. Special consideration was taken to account for the dependency created because each participant contributed observations in two of the four possible training conditions and at multiple time points. ANOVA models were estimated using covariance pattern models. Two different error covariance structures were compared prior to hypothesis testing: compound symmetry and unstructured. Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (BIC) values were compared to determine the most appropriate model. If there was a significant time x condition interaction ($p \leq 0.05$), we examined simple effects. Otherwise, main effects of time and condition were examined. A one-factor analysis of variance (ANOVA) was used to detect differences across time for control muscle thickness. Results are presented as mean (SE) unless otherwise stated.

RESULTS

Demographics

At baseline, participants (n = 40) had a mean (SD) age of 21 (2) years, height of 171.56 (9.32) cm, body mass of 68.37 (11.49) kg, and BMI of 23.14 (2.83) kg/m^2.

Muscle Thickness

There was no difference (mean, 95% CI) across time for control muscle thickness [0.07 (−0.006, 0.151); p = 0.054]. There were no time × condition interactions for anterior (30%, p = 0.607; 40%, p = 0.828; 50%, p = 0.782; 60%, p = 0.740) or lateral (30%, p = 0.492; 40%, p = 0.656; 50%, p = 0.414; 60%, p = 0.354) muscle thickness sites. There was a main effect of time for all anterior (all $p < 0.001$; **Figure 1**) and lateral (all $p < 0.001$; **Figure 2**) muscle thickness sites, which increased in response to training. There was also a main effect of condition for all sites (70/0 greater than all other conditions, all $p \leq 0.007$) except the 30% lateral site (p = 0.058). Changes in anterior and lateral muscle thickness separated by condition can be seen in **Tables 1**, **2**, respectively.

One-Repetition Maximum

A time x condition interaction for 1RM ($p < 0.001$; **Figure 3**) showed the response to training was greater in 70/0 compared to 15/0 [mean difference = 3.2 (0.7) kg, $p < 0.001$], 15/40 [mean difference = 3.0 (0.7) kg, $p < 0.001$], and 15/80 [mean difference = 2.4 kg (0.7), p = 0.002]. 70/0 increased 1RM from baseline [29.4 (1.3) to 32.6 (1.3) kg, $p < 0.001$], while 15/0 [30.3 (1.3) to 30.2 (1.3) kg, p = 0.913], 15/40 [30.0 (1.3) to 30.1 (1.3) kg, p = 0.909), and 15/80 [28.6 (1.3) to 29.3 (1.3) kg, p = 0.220] did not.

Isometric and Isokinetic Strength

There was no time x condition interaction for isometric strength (p = 0.292) or isokinetic strength at 60°/s (p = 0.537) and 180°/s (p = 0.180). There was a main effect of time for isometric strength (219.5 (10.3) to 230.1 (10.3) Nm, p = 0.002) and isokinetic strength at 180°/s [139.2 (6.8) to 147.8 (6.8) Nm, $p < 0.001$], while isokinetic strength at 60°/s did not change [198.0 (8.0)

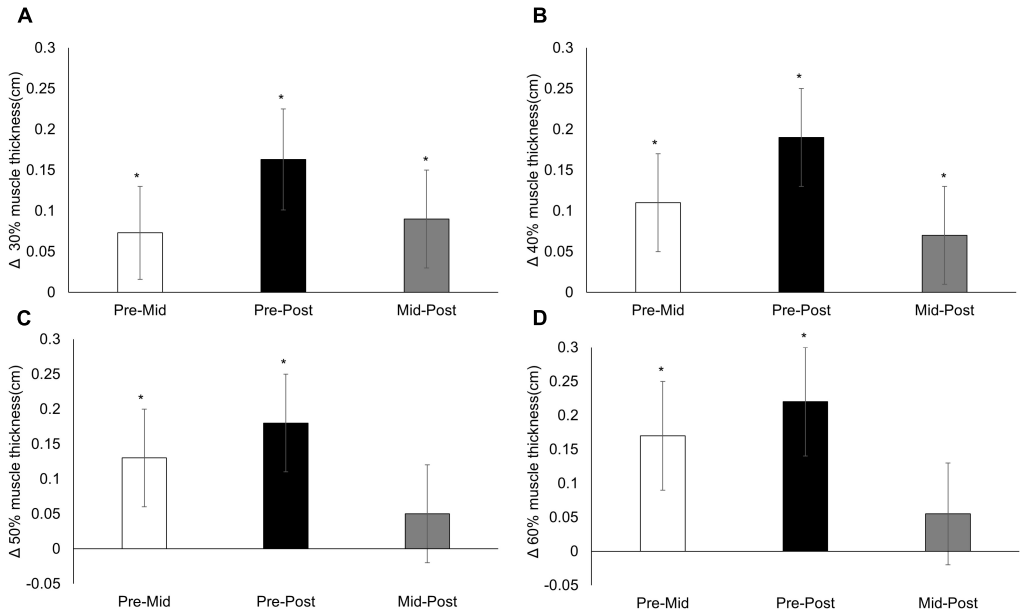

FIGURE 1 | Anterior muscle thickness. Changes from pre-training, mid-training, and post-training for anterior muscle thickness at **(A)** 30%, **(B)** 40%, **(C)** 50%, and **(D)** 60% of the upper leg, collapsed across conditions. Data presented as mean changes (95% CI). An * indicates a significant change from pre. Alpha level = 0.05.

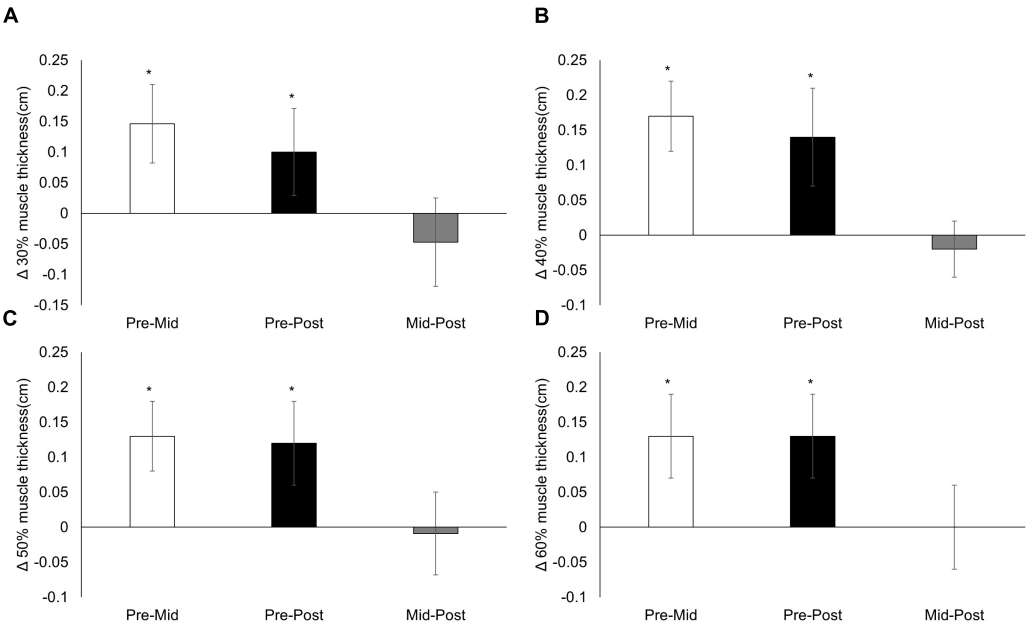

FIGURE 2 | Lateral muscle thickness. Changes from pre-training, mid-training, and post-training for lateral muscle thickness at **(A)** 30%, **(B)** 40%, **(C)** 50%, and **(D)** 60% of the upper leg, collapsed across conditions. Data presented as mean changes (95% CI). An * indicates a significant change from pre. Alpha level = 0.05.

to 200.5 (8.0) Nm, $p = 0.261$]. Changes for dynamometry are depicted in **Figure 4** and can be seen separated by condition in **Table 3**.

Endurance

A time x condition interaction for endurance repetitions ($p = 0.028$; **Figure 5**) showed the increase in repetitions for 15/80 was greater compared to 15/0 [mean difference = 1.9 (0.7) repetitions, $p = 0.014$] and 70/0 [mean difference = 2.1 (0.7) repetitions, $p = 0.006$]. Endurance repetitions increased for all conditions: 15/0 = 20 (1.1) to 24 (0.9) repetitions, $p < 0.001$; 15/40 = 21 (1.1) to 25 (1.0) repetitions, $p < 0.001$; 15/80 = 21 (1.1) to 27 (0.9) repetitions, $p < 0.001$; 70/0 = 22 (1.1) to 26 (0.9) repetitions, $p < 0.001$.

TABLE 1 | Anterior muscle thickness changes per condition (cm).

Site	Change	15/0	15/40	15/80	70/0
30%	pre-mid*	0.11 (−0.01, 0.22)	0.00 (−0.13, 0.12)	0.08 (−0.04, 0.20)	0.10 (−0.01, 0.23)
	pre-post*	0.19 (0.07, 0.31)	0.05 (−0.07, 0.18)	0.17 (0.05, 0.30)	0.22 (0.09, 0.34)
40%	pre-mid*	0.13 (0.01, 0.26)	0.11 (−0.01, 0.24)	0.04 (−0.08, 0.17)	0.16 (0.03, 0.29)
	pre-post*	0.20 (0.08, 0.32)	0.14 (0.01, 0.27)	0.19 (0.06, 0.32)	0.22 (0.09, 0.35)
50%	pre-mid*	0.16 (0.03, 0.29)	0.17 (0.04, 0.31)	0.03 (−0.09, 0.17)	0.16 (0.02, 0.29)
	pre-post*	0.19 (0.06, 0.32)	0.18 (0.05, 0.32)	0.17 (0.04, 0.31)	0.20 (0.06, 0.33)
60%	pre-mid*	0.22 (0.07, 0.37)	0.19 (0.04, 0.35)	0.05 (−0.10, 0.21)	0.21 (0.05, 0.37)
	pre-post*	0.23 (0.08, 0.38)	0.21 (0.05, 0.37)	0.20 (0.04, 0.36)	0.25 (0.09, 0.41)

Changes (cm) in muscle thickness from pre to mid-training and pre to post-training for anterior sites on the upper leg separated by condition. Data presented as mean change (95% CI). An asterisk next to change label indicates a main effect of time, whereby all conditions changed similarly within the respective time period. There were no significant changes between conditions within each time frame. Alpha level = 0.05.

TABLE 2 | Lateral muscle thickness changes per condition (cm).

Site	Time	15/0	15/40	15/80	70/0
30%	pre-mid*	0.10 (−0.03, 0.24)	0.04 (−0.10, 0.18)	0.22 (0.08, 0.37)	0.20 (0.06, 0.35)
	pre-post*	0.10 (−0.02, 0.24)	0.02 (−0.12, 0.17)	0.17 (0.03, 0.32)	0.08 (−0.05, 0.23)
40%	pre-mid*	0.19 (0.11, 0.28)	0.16 (0.07, 0.25)	0.10 (0.02, 0.19)	0.21 (0.12, 0.30)
	pre-post*	0.16 (0.07, 0.26)	0.13 (0.03, 0.23)	0.09 (−0.00, 0.19)	0.19 (0.09, 0.29)
50%	pre-mid*	0.08 (−0.02, 0.20)	0.17 (0.05, 0.29)	0.03 (−0.08, 0.15)	0.22 (0.10, 0.34)
	pre-post*	0.12 (0.00, 0.23)	0.12 (0.00, 0.24)	0.07 (−0.04, 0.19)	0.15 (0.03, 0.27)
60%	pre-mid*	0.13 (0.01, 0.24)	0.22 (0.09, 0.34)	0.01 (−0.10, 0.13)	0.17 (0.05, 0.29)
	pre-post*	0.10 (−0.01, 0.21)	0.18 (0.06, 0.30)	0.10 (−0.02, 0.22)	0.15 (0.03, 0.27)

Changes (cm) in muscle thickness from pre to mid-training and pre to post-training for lateral sites on the upper leg separated by condition. Data presented as mean change (95% CI). An asterisk next to change label indicates a main effect of time, whereby all conditions changed similarly within that time period. There were no significant changes between conditions within each time frame. Alpha level = 0.05.

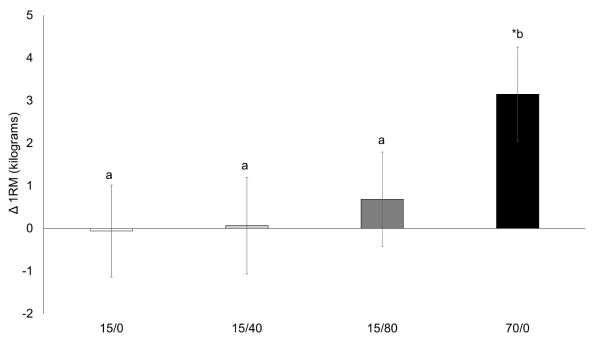

FIGURE 3 | Knee extension 1RM. Changes from pre-training to post-training knee extension 1RM (one-repetition maximum) for each condition. Data presented as mean changes (95% CI). An * indicates a significant change from pre. Letters indicate significant differences between conditions. If conditions share the same letter they are not different from one another. Alpha level = 0.05.

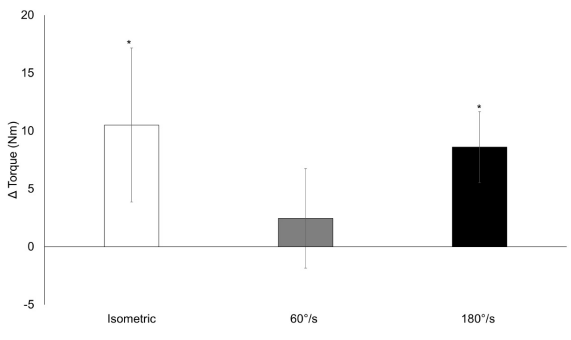

FIGURE 4 | Isometric and isokinetic torque. Changes from pre-training to post-training for isometric and isokinetic strength at 60°/s and 180°/s collapsed across conditions. Data presented as mean changes (95% CI). An * indicates a significant change from pre. Alpha level = 0.05.

Exercise-Induced Swelling

There was no time x condition interaction for the muscle swelling response (p = 0.574). There was, however, a main effect of time (p < 0.001; **Figure 6**) and condition (p < 0.001). For all conditions, the muscle swelling response increased from training session 1 to session 9 [0.14 (0.03) cm, p < 0.001] and again from session 9 to session 15 [0.06 (0.03) cm, p = 0.042). Collapsed across time, 15/0 elicited the greatest swelling response (all $p \leq 0.014$), and 15/40 was greater than 15/80 (p = 0.011).

Exercise Volume

Weekly exercise volume was calculated as the average number of repetitions completed over the two weekly training sessions, multiplied by the load lifted. There was a time x condition interaction for weekly exercise volume (p < 0.001; **Table 4**). In general, volume increased in most successive weeks, for

TABLE 3 | Isometric and isokinetic strength changes per condition (Nm).

MVC	15/0	15/40	15/80	70/0
Isometric*	−0.50 (− 13.45, 12.45)	13.99 (0.37, 27.61)	13.15 (− 0.12, 26.43)	15.42 (2.14, 28.70)
60°/s	−2.01 (− 10.39, 6.37)	3.99 (− 4.81, 12.80)	1.29 (− 7.29, 9.88)	6.52 (− 2.06, 15.11)
180°/s*	6.92 (0.91, 12.93)	5.94 (− 0.38, 12.28)	7.07 (0.91, 13.24)	14.51 (8.45, 20.57)

Changes (Nm) from pre to post-training in isometric and isokinetic (60°/s and 180°/s) maximal voluntary contraction (MVC) separated by condition. Data presented as mean change (95% CI). An asterisk next to MVC label indicates a main effect of time, whereby all conditions changed similarly from baseline. There were no significant differences between conditions for any measure. Alpha level = 0.05.

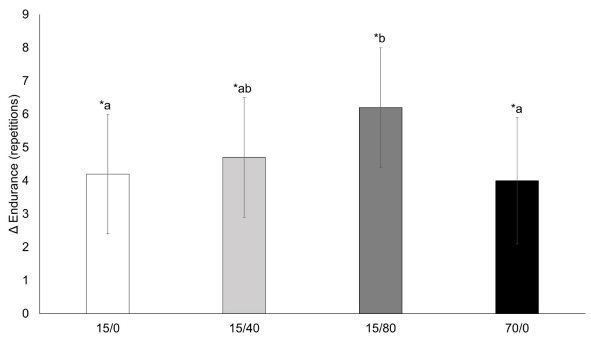

FIGURE 5 | Knee extension endurance. Changes from pre-training to post-training knee extension endurance repetitions for each condition. Data presented as mean changes (95% CI). An * indicates a significant change from pre. Letters indicate significant differences between conditions. If conditions share the same letter they are not different from one another. Alpha level = 0.05.

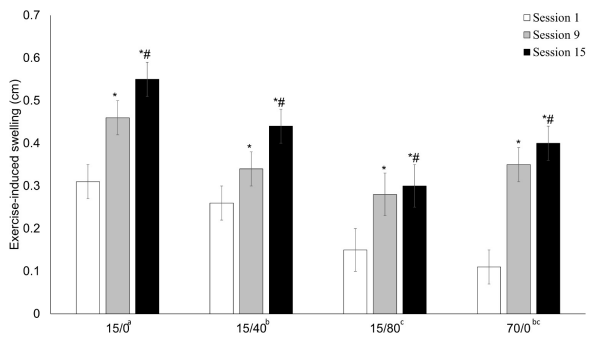

FIGURE 6 | Exercise-induced swelling response. The exercise-induced swelling response to session 1, session 9, and session 15. Data presented as mean (SE). An * indicates a significant difference from session 1, whereas # indicates a significant difference from session 9. Letters next to condition labels indicate significant differences (main effect of condition). If conditions share the same letter they are not different from one another. Alpha level = 0.05.

each condition, throughout the training protocol. In week 1, 15/40, and 70/0 did not differ in volume (p = 0.365) nor did 15/0 and 70/0 in weeks 2, 5, 6, 7, and 8 (all $p \geq 0.183$). All other conditions differed from one another when comparing them in each remaining week (all $p \leq 0.021$). Two participants, using condition 15/0, reached 360 goal repetitions, one at session 14 the other at session 5. Both completed all repetitions for the remaining sessions (**Figure 7**).

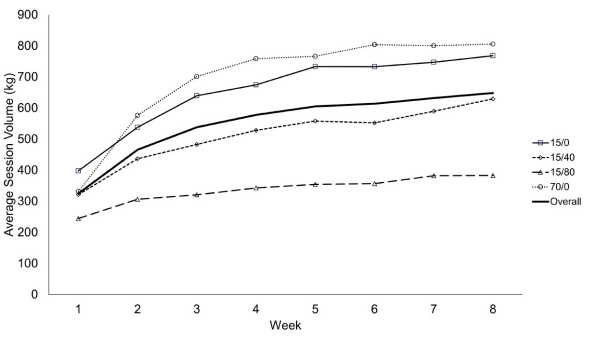

FIGURE 7 | Average training volume per session during each week of the training protocol. Data presented as mean values. This figure is a visual aid, and as such, no statistical comparisons are denoted.

All others reached volitional failure at some point during the protocol.

DISCUSSION

The main findings of the current study were that 1RM changes favored the high-load condition, while isometric and isokinetic strength responded similarly across all conditions. The increase in endurance was greater in the 15/80 condition compared to 15/0 and 70/0. The increase in muscle thickness from training seemed to be uniform across conditions. We believe the changes in muscle thickness were due to muscle growth rather than edema as there was an exercise-induced swelling response at training sessions 1, 9, and 15, suggesting minimal swelling prior to exercise at each phase of training. Training volume was greatest in non-restriction conditions and then decreased with increased pressure.

Strength

When comparing strength outcomes to Holm et al. (2008) who also compared 15 and 70% 1RM, and a recent meta-analysis by Lixandrão et al. (2018) we found similar results in that changes in 1RM favor high-loads over low-loads. However, we diverge from Holm et al. (2008) regarding our low-load conditions, which did not increase 1RM in the current study. The responses in 1RM, favoring high-load over very low-load, were expected due to the practicing of a skill that more closely resembles this specific strength test (Buckner et al., 2017b). To illustrate, previous studies have found that changes in 1RM favor high-load

TABLE 4 | Average weekly exercise volume per session (kg).

Week	15/0	15/40	15/80	70/0	Condition
1	403.2^{a}	315.2^{a}	214.2^{a}	329.3^{a}	15/0 vs. all; 15/80 vs. all
2	533.5^{b}	436.1^{b}	278.2^{b}	568.8^{b}	15/0 vs. 15/40, 15/80; 15/40 vs. all; 15/80 vs. all
3	637.2^{c}	492.3^{c}	298.9^{b}	704.5^{c}	15/0 vs. all; 15/40 vs. all; 15/80 vs. all
4	676.4^{d}	537.1^{d}	320.7^{c}	762.8^{de}	15/0 vs. all; 15/40 vs. all; 15/80 vs. all
5	732.2^{e}	582.1^{ef}	343.4^{de}	756.6^{d}	15/0 vs. 15/40, 15/80; 15/40 vs. all; 15/80 vs. all
6	745.8^{e}	571.5^{e}	339.3^{cd}	790.7^{ef}	15/0 vs. 15/40, 15/80; 15/40 vs. all; 15/80 vs. all
7	764.1^{f}	610.5^{f}	367.1^{e}	803.8^{fg}	15/0 vs. 15/40, 15/80; 15/40 vs. all; 15/80 vs. all
8	799.2^{g}	611.0^{f}	365.2^{de}	825.9^{g}	15/0 vs. 15/40, 15/80; 15/40 vs. all; 15/80 vs. all

Average training volume (kg) per session during each week (1–8) of the training protocol. Data presented as mean. Letters indicate significant differences between weeks within each condition. If at least one letter is similar there are no differences between weeks. Condition column indicates significant differences between listed conditions within each week. If not listed conditions are not different from each other. Alpha level = 0.05.

training over low-loads (Mitchell et al., 2012; Lasevicius et al., 2018), yet when a low-load group periodically practices the 1RM test the differences are diminished (Morton et al., 2016). During the study by Holm et al. (2008), 1RM was assessed (practiced) every 10th training session, which may explain the difference from our low-load conditions as our participants were only tested pre and post, thus having minimal practice of the test.

We found no effect of load or BFR on dynamometry measured strength changes while Holm et al. (2008) found a favorable change for high loads. BFR has been previously shown to augment the isometric (Shinohara et al., 1998) and 1RM (Laurentino et al., 2012) strength response to low-load training, but that was not observed in the current study. Kacin and Strazar (2011) also found no effect of very low-load training (15% maximum strength) with or without restriction on isometric strength changes. In comparison with previous studies that used 20% 1RM (Laurentino et al., 2012) and 40% (Shinohara et al., 1998) maximum strength respectively, training with 15% 1RM, regardless of restriction, may be too low to induce a meaningful increase in maximal isometric or isokinetic strength. This suggests either a potential loading threshold, or a lack of practice with the test overall as we only performed dynamometry pre and post-training. While we did find a time effect for improvements in isometric and isokinetic strength at 180°/s, they were small relative to the movement and the population tested (young healthy adults). Future research should seek to compare the response to these conditions in clinical populations to determine the effect on muscle strength. While the relative improvement in this study was low, the effect could be greater or more meaningful in those with limited physical function. Further if BFR reduces the workload while providing similar muscular improvements it could be an effective therapeutic tool.

Endurance

While endurance improved across all conditions it was augmented over 15/0 and 70/0 by combining a very low-load with BFR at 80% AOP. While Holm et al. (2008) did not measure endurance as an outcome, Kacin and Strazar (2011) found that BFR training augmented endurance over an equally loaded, non-restricted group. The mechanism causing this greater response to endurance may either be strictly physiological, psychophysiological, or both. During resistance exercise with 20% 1RM, greater BFR pressure (i.e., 230 mmHg) augments the metabolic stress compared to BFR with 180 mmHg and a non-restriction condition (Sugaya et al., 2011). BFR also augments angiogenic gene expression in response to acute low-load resistance exercise (Larkin et al., 2012; Ferguson et al., 2018). Over a chronic training period the adaptation to 15/80 may have reflected the greater metabolic disturbances and angiogenic gene expression within the muscle, thus increasing the capacity to deal with a metabolic disturbance and/or result in a greater capillarization compared to 15/0 and 70/0. In addition, psychophysiological adaptations may have also occurred. For example, as greater BFR pressures (i.e., 80% AOP) are associated with greater perceptions of exertion and discomfort at very low-loads (Jessee et al., 2017; Dankel et al., 2018), the participants training with 80% AOP may have become accustomed to these feelings and more able to withstand similar feelings during an endurance test to volitional failure, resulting in more repetitions. The increased endurance from pre to post-training in all conditions is supported by the increased weekly volume during training which could reflect an increased work capacity. Although the exact mechanism was not explored in the current study, it seems as though greater BFR pressure (i.e., 80% AOP) augments the improvement in endurance over very low loads alone. This finding could have positive implications for clinical and elderly populations as activities of daily living are often submaximal and repetitive; however, future research should examine if these improvements in knee extension endurance do indeed transfer to activities of daily living.

Muscle Thickness and Swelling

Our findings of similar improvements in muscle thickness across all sites, regardless of condition, differ from those of Holm et al. (2008) and Lixandrao et al. (2015). We believe the discrepancies can be explained by exercising to volitional failure. While on average our participants exercised to volitional failure (only 2 participants reached 90 repetitions for all four sets), Holm et al. (2008) work matched the low-load condition to high-load, and Lixandrao et al. (2015) used an arbitrary, though

commonly used, repetition protocol (3 sets of 15 repetitions). Therefore, in the Holm et al. (2008) and Lixandrao et al. (2015) studies the low-load conditions were likely stopped prior to failure, whereas the high-load conditions were at or near failure, meaning more muscle fibers would be stimulated for hypertrophy (Dankel et al., 2016). While it seems the data by Lixandrao et al. (2015) supports the need of a greater restriction pressure (80% AOP vs. 40% AOP) to augment muscle growth, it may only be working indirectly by causing fatigue to occur earlier (Ganesan et al., 2015), within the allotted goal repetitions. We argue that, were exercise performed to volitional failure, the muscle growth may have been similar across conditions. In fact, multiple experimental studies have shown that when comparing low-loads with and without BFR (Fahs et al., 2015; Farup et al., 2015), as well as low-loads and high-loads (Mitchell et al., 2012; Morton et al., 2016), when taken to failure muscle growth is similar. Overall, the current data suggests that when exercising to volitional failure the increase in muscle size is neither load nor pressure dependent, supporting a recent meta-analysis resulting in a similar conclusion (Lixandrão et al., 2018). In contrast, Lasevicius et al. (2018) conclude that there is a loading threshold between 20 and 40% 1RM that must be surpassed to optimize muscle growth. While these differences could be due to differing image analyses (the authors used separate muscle thickness images to estimate cross-sectional area), the data from Lasevicius et al. (2018) could also suggest that when equated for volume greater external loads will provide a more robust stimulus per repetition. Regardless, their finding of a need to use greater loads to maximally stimulate muscle growth is not a consistent finding and more work should be done to reconcile these differences.

As concerns exist regarding the ability to distinguish true muscle growth from muscle edema in the early phases of a training program (Damas et al., 2016), we sought to investigate the ability of the muscle to swell in response to exercise over the course of the training protocol. A previous investigation found a limit in the ability for exercise to induce muscle swelling by showing that a swollen muscle did not swell further when undergoing a second bout of exercise (Buckner et al., 2017a). We found that the exercise-induced swelling response was present at each time-point and increased over the training protocol. The increase in the swelling response across time may have been due to the increase in exercise volume across the training protocol, or perhaps the increase in muscle size, which could theoretically hold a greater volume of fluid. A previous study measuring acute exercise-induced changes in muscle thickness at the beginning and end of a training program, found a muscle swelling response at both time points (Farup et al., 2015). Thus, we believe we were measuring true muscle growth with our muscle thickness measurements, as a damaged/swollen muscle likely would not have responded to exercise-induced swelling.

Exercise Volume

Despite the magnitude of difference in loads, 15/0 and 70/0 did not differ across most weeks. By design, repetitions were limited to 90/set, therefore, the volume was potentially limited in the 15/0 condition. We believe this generally only affected volume in the early sets as only 2 of 40 total participants eventually reached 360 repetitions during training. Although it was not required to reach volitional failure, and did not seem to augment the strength or muscle size response to very-low loads, BFR did reduce the amount of volume required to elicit adaptations in a pressure dependent manner. This may be important for a variety of populations that wish to increase muscle size and endurance but wish to limit the amount of overall work whether it be due to injury, frailty, or a simple desire to limit joint stress. In fact, very low-load resistance with 15% 1RM may more closely resemble the ability of some clinical populations rather than the more oft used 30% 1RM in low-load training protocols. Furthermore, while volume is thought to be an important training variable, the data herein suggests there may not be a dose-response relationship with respect to muscle growth, as all conditions increased muscle thickness similarly. Thus, there is likely a point where additional volume is no longer augmenting muscle growth. Future research should investigate whether a minimum volume threshold to elicit adaptation exists and whether or not that threshold differs between trained and untrained individuals.

Limitations

Our study may have been limited by the design, requiring each participant to train unilaterally using two different conditions, potentially inducing a crossover effect between legs. However, these issues are likely minimal as both limbs were trained (MacInnis et al., 2017) and we were able to detect differences in strength changes. Our statistical analysis also helped to account for any potential issues of dependency (two conditions from each participant). BFR was based upon a percentage of resting AOP measurement, rather than quantified blood flow, thus, no assumptions can be made about the actual percent reduction in blood flow caused by the different cuff inflation pressures. While the amount of work increased most weeks, the difference in 1RM changes between conditions might have been greater had loads been progressed, however, doing so could have posed a separate set of limitations. For example, we were also attempting to determine if BFR could augment a training load that was perhaps too low for adaptation, had 15% 1RM conditions been progressed it would have limited the ability to elucidate whether adaptation was due to progressed load or BFR. Further, we felt that progressing the load in 70/0 and not the other conditions would create a greater limitation to the specific aims of the study. Also, the rest periods between sets for 15% conditions were much shorter than 70%, meaning the training density differed between conditions, likely influencing volume completed. However, rest periods used were in accordance with commonly used BFR and traditional high load protocols. Lastly, for a measure of reliability, we included a control muscle thickness site on an upper body muscle group that was not trained. Including muscle thickness measures on the leg for a time matched non-exercise control group would have been a stronger design.

Conclusion

An increase in strength was seen in the 1RM test following high-load training only and there were no differences in conditions for isometric or isokinetic measures suggesting that increases in isotonic strength are load dependent. The increase in an unpracticed or "general strength" test did not differ due to load, suggesting that the effect of load is task specific and may not translate to other tasks well, however, this may require further research to confirm. The current data also suggest that the application of a higher BFR pressure creates a unique stimulus compared to non-restriction conditions to increase endurance. Muscle size did not depend on load, nor was it affected by the differences in volumes or restriction pressures. Given muscle size increases did not differ across conditions, despite differences in exercise volume, suggests a lack of a dose-response relationship. Furthermore, the lack of strength increase in the very low-load conditions while similar increases in muscle size were found suggests a dissociation between the two.

AUTHOR CONTRIBUTIONS

MJ, SB, JM, KM, SD, TA, ZB, and JL were involved with conceptualization, implementation, and data collection. MJ, JB, and JL were involved with statistical analyses. MJ, SB, JM, KM, SD, TA, ZB, JB, and JL were involved with drafting and editing the manuscript.

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Effect of Ischemic Preconditioning on the Recovery of Cardiac Autonomic Control From Repeated Sprint Exercise

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Repeated sprint exercise (RSE) acutely impairs post-exercise heart rate (HR) recovery (HRR) and time-domain heart rate variability (i. e., RMSSD), likely in part, due to lactic acidosis-induced reduction of cardiac vagal reactivation. In contrast, ischemic preconditioning (IPC) mediates cardiac vagal activation and augments energy metabolism efficiency during prolonged ischemia followed by reperfusion. Therefore, we investigated whether IPC could improve recovery of cardiac autonomic control from RSE partially via improved energy metabolism responses to RSE. Fifteen men team-sport practitioners (mean ± SD: 25 ± 5 years) were randomly exposed to IPC in the legs (3 × 5 min at 220 mmHg) or control (CT; 3 × 5 min at 20 mmHg) 48 h, 24 h, and 35 min before performing 3 sets of 6 shuttle running sprints (15 + 15 m with 180° change of direction and 20 s of active recovery). Sets 1 and 2 were followed by 180 s and set 3 by 360 s of inactive recovery. Short-term HRR was analyzed after all sets via linear regression of HR decay within the first 30 s of recovery (T30) and delta from peak HR to 60 s of recovery (HRR60s). Long-term HRR was analyzed throughout recovery from set 3 via first-order exponential regression of HR decay. Moreover, RMSSD was calculated using 30-s data segments throughout recovery from set 3. Energy metabolism responses were inferred via peak pulmonary oxygen uptake ($\dot{V}O_2$peak), peak carbon dioxide output ($\dot{V}CO_2$peak), peak respiratory exchange ratio (RERpeak), first-order exponential regression of $\dot{V}O_2$ decay within 360 s of recovery and blood lactate concentration ([Lac-]). IPC did not change T30, but increased HRR60s after all sets (condition main effect: $P = 0.03$; partial eta square (η^2_p) = 0.27, i.e., large effect size). IPC did not change long-term HRR and RMSSD throughout recovery, nor did IPC change any energy metabolism parameter. In conclusion, IPC accelerated to some extent the short-term recovery, but did not change the long-term recovery of cardiac autonomic control from RSE, and such accelerator effect was not accompanied by any IPC effect on surrogates of energy metabolism responses to RSE.

Keywords: ischemia, supramaximal exercise, parasympathetic, heart beat, metabolism

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INTRODUCTION

Training with repeated sprint exercise (RSE) requires less time per session (Stork et al., 2017) and induces similar or even superior feelings of satisfaction (Stork et al., 2017) and aerobic adaptations (Gist et al., 2014) compared to continuous endurance training. Sprint-based training has therefore been considered as a valuable strategy to improve health-related physical fitness of subjects with little time available to engage in regular programs of exercise training (Gist et al., 2014; Stork et al., 2017). However, heart rate (HR) recovery (HRR) is acutely slower and recovery of time domain heart rate variability (HRV) is acutely blunted post-RSE as compared with post-moderate continuous exercise matched to net energy expenditure (Buchheit et al., 2007; Nakamura et al., 2009; Del Rosso et al., 2017). Of note, the acute slowing of HRR after RSE may raise clinical concerns, because slow HRR from maximal exercise, particularly within the first 60 s of recovery (i.e., short-term recovery), is strongly associated with increased risk of cardiac events in subjects with cardiovascular risk factors (Cole et al., 1999).

Post-exercise HRR and HRV are assumed to be mostly determined by interplay between cardiac vagal reactivation and sympathetic withdrawal to the sinus node (Goldberger et al., 2006; Coote, 2010; Peçanha et al., 2014). Thus, methods that improve the cardiac autonomic control could possibly attenuate the acute effect of RSE on post-exercise HRR and HRV. In this context, non-lethal brief cycles of ischemia-reperfusion [i.e., ischemic preconditioning (IPC)] at a site (e.g., limb) induce powerful protection against injury provoked by prolonged ischemia and subsequent reperfusion at a remote site (e.g., heart) (Kharbanda et al., 2002), and the vagal branch of the autonomic nervous system seems to play a pivotal role in such IPC-mediated protection. The reason is that, in rats, vagotomy (Basalay et al., 2012), blockade of muscarinic receptors with atropine (Mastitskaya et al., 2012) or optogenetic silencing of vagal pre-ganglionic neurons (Mastitskaya et al., 2012) nullified the IPC protection against ischemia-reperfusion injury. In addition, IPC not only protects against injury, but may improve some healthy phenotypes (Cocking et al., 2018; Jeffries et al., 2018). For instance, IPC increased resting high-frequency HRV (i.e., a surrogate of cardiac vagal control) in healthy uninjured men (Enko et al., 2011). Therefore, evidence from both ischemia-reperfusion models and healthy resting humans supports that IPC exerts an excitatory effect on the vagal branch of the autonomic nervous system. However, it remains untested whether IPC accelerates post-exercise HRR and augments post-exercise time domain HRV, particularly during the short-term recovery period, in which HRR and time domain HRV are mostly determined by the vagal control of the heart. Testing this issue in healthy humans could then motivate further studies in subjects at risk for cardiovascular events, in case the IPC shows a promising beneficial effect.

If IPC improves post-exercise HRR and time-domain HRV, the IPC effect could be mediated by two non-excluding possibilities. On the one hand, mechanisms that mediate the IPC vagal excitation in models of ischemia-reperfusion injury could play a role (Gourine and Gourine, 2014). These mechanisms include activation of afferent pain fibers at the preconditioned site, as well as release of substances in the circulation from the preconditioned site (Gourine and Gourine, 2014). Thus, one plausible hypothesis is that neural and humoral mechanisms triggered at the preconditioned site could directly activate cardiac vagal neurons leading to a possible beneficial effect of IPC on post-exercise HRR and time domain HRV. On the other hand, IPC could improve post-exercise HRR and time domain HRV via reduction of the exercise-induced energy metabolism distress, which could indirectly increase the cardiac vagal control. Two sets of evidence support such energy metabolism hypothesis. Firstly, manipulation of the aerobic and anaerobic lactic energy contribution to the total energy expenditure of exercise showed that the lower the contribution of the anaerobic lactic metabolism, the faster is the HRR (Buchheit et al., 2007; Nakamura et al., 2009; Del Rosso et al., 2017). The underlying reason for this phenomenon is unknown, but it may involve less metabolites buildup leading to less activation of metabolite-sensitive receptors like muscle metaboreceptors and carotid chemoreceptors (Buchheit et al., 2007). Secondly, IPC augments energy metabolism efficiency in skeletal muscles of pigs exposed to prolonged ischemia (Pang et al., 1995), presumably due to increased efficiency of the mitochondrial electron transport chain (Garlid et al., 2003; Thaveau et al., 2007; Cabrera et al., 2012). Furthermore, some studies have reported IPC to increase peak pulmonary oxygen uptake ($\dot{V}O_2$) (de Groot et al., 2010; Cruz et al., 2015) and decrease blood lactate concentration [Lac-] (Bailey et al., 2012) during incremental dynamic exercise in moderately trained subjects, suggesting increased aerobic and decreased anaerobic lactic contribution to exercise energy metabolism. Others have reported IPC not to modify energy metabolism responses to RSE (Patterson et al., 2015; Griffin et al., 2018), but perhaps the IPC dose (1-day exposure) that was effective for incremental dynamic exercise may not be sufficient for RSE. Herein, we chose to test the later hypothesis (i.e., energy metabolism hypothesis) and we employed an IPC dose (3-day exposure) greater than previous studies that investigated the IPC effect on energy metabolism responses to RSE (Patterson et al., 2015; Griffin et al., 2018). Thus, we investigated whether repeated exposure to IPC could improve recovery of cardiac autonomic control from RSE partially via improved energy metabolism responses to RSE.

METHODS

Subjects

Fifteen healthy men participated in the study (mean ± standard deviation: 25 ± 5 years, 81.2 ± 9.7 kg and 179.3 ± 7.4 cm). Subjects were engaged in some type of physical training at least three times a week during the last year and in non-professional team sport competitions (e.g., soccer and basketball). All subjects provided written informed consent before participating in the study. The study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Federal University of São Paulo (process number: 192.224).

Experimental Design

The study was single-blinded, crossed-over, randomized and controlled. Each subject visited the laboratory on seven occasions (**Figure 1**). Just one subject was assessed at a time. The first visit was used for familiarization and measurement of the best time in a 30-m sprint, with a change of direction of 180° at the middle of the course (i.e., 15 + 15 m). Subjects had three to six trials, separated by 180 s of inactive recovery, to achieve their best time (BT). Then, the BT served as reference in the RSE task to check an all-out pacing strategy. Exposure to IPC and CT occurred on three occasions, 48 and 24 h before the RSE task, as well as on the day of the RSE task. The last exposure to IPC and CT finished 35 min before the onset of the RSE task. Likewise previous studies (de Groot et al., 2010; Crisafulli et al., 2011; Bailey et al., 2012; Barbosa et al., 2015; Cruz et al., 2015; Del Rosso et al., 2017), subjects were not informed about the study hypothesis in an attempt of blinding. RSE was performed in a sports court, at the same time of the day, with interval between each test of at least 7 and most 14 days. Subjects were instructed to eat a light meal 2 h before RSE, as well as not to train and consume caffeine and alcohol 24 h before RSE.

Ischemic Preconditioning and Control

IPC was performed with subjects seated using customized cuffs which were specifically made to occlude the circulation at the tights (Ferreira et al., 2016). One cuff was used per tight. The cuff had two independent bladders mounted in series. Each bladder was 36 cm long and 17.5 cm wide. Together the bladders covered at least 80% of a thigh's circumference. An aneroid manometer and an inflation bulb were attached to each bladder. Each cuff had a Velcro strap which, in most cases, surrounded a tight at least two times. In the IPC procedure, cuffs were inflated one at a time to 220 mmHg for 5 min. A total of three inflation/deflation cycles were performed per leg (de Groot et al., 2010). Cuff inflation generally took 15–25 s. Occlusion time started to be counted after the target pressure was achieved. In the CT procedure, cuffs were inflated to 10 mmHg for 5 min and deflated to 0 mmHg for 5 min.

The target IPC inflation pressure of 220 mmHg should be more than enough to cause arterial and venous occlusion in the tights of normotensive subjects using large cuffs as ours. Even so, we used a vascular Doppler (Doppler vascular 610B, MEDMEGA, Brazil) to guarantee the presence of arterial occlusion. The site that yielded the best blood flow signal at the posterior tibial artery was identified at the beginning of each experimental day. The skin was marked at this site. The head of the Doppler transducer was positioned at the marked site at pre-inflation and maintained at this place while a cuff was inflated. The signal usually disappeared when the cuff pressure achieved 140–160 mmHg. Thus, at the target pressure no flow signal was present in any inflation in any subject.

IPC yields two windows of protection against ischemia-reperfusion injury (Hausenloy and Yellon, 2010). The first begins immediately after the IPC exposure and lasts about 2 h (Hausenloy and Yellon, 2010). The second begins approximately 12–24 h after the IPC exposure and can last for 48–72 h (Hausenloy and Yellon, 2010). Of note, repeated exposure to IPC before the second window effect is over has been shown to amplify the IPC effect on the resting vascular function in humans (Loukogeorgakis et al., 2005; Jones et al., 2014). Although an effective dose and timing of IPC administration has not yet been determined for the sake of improvement of exercise-related responses, evidence indicates that the IPC ergogenic effect may last for many hours (Lisbôa et al., 2017). Hence, in the present study, IPC was applied on two consecutive days before, as well as on the day of the RSE task in an attempt to sum early and late effects of IPC (Loukogeorgakis et al., 2005), which could amplify the IPC effect (Jones et al., 2014). However, the use of this design precluded identification of the effective dose of IPC (i.e., the effects from just the acute or repeated exposure could be responsible).

Repeated Sprint Exercise

The warm-up began 10 min after IPC or CT exposure. The warm-up consisted of a standardized and supervised exercise routine that lasted 15 min, including moderate intensity running (5 min), athletic drills (Anfersen and Skipping), dynamic stretching and four maximal accelerations (15 m) with a change of direction at the end (180°), separated by 1 min of active recovery (walking). Once the warm-up ended, a portable metabolic analyzer (K4b2, Cosmed, Italy) was placed in the subjects, which usually took approximately 10 min.

The RSE task consisted of three sets (i.e., bouts) of six sprints. Each sprint was 30-m long, with a change of direction of 180° at the middle of the course (15 + 15 m). There were 20 s of active recovery between sprints, 180 s of inactive recovery between sets and 360 s of inactive recovery after the last set. During the active recovery, subjects slowed down (10 m), scrolled through a 24-m course during 15–17 s, and then waited for 3–5 s at the start position for the sound signal of the next sprint (**Supplementary Video 1**). Sound signals were automatically emitted by a photocell system (Test Speed 6.0, CEFISE, Brazil). Pace at the recovery course was verbally informed in order to maintain a correct scroll rhythm. At the end of each set, subjects rapidly slowed down and sat on a chair positioned beside the recovery course (10 m after the finish line).

Subjects were instructed to run as fast as possible during every sprint and were verbally encouraged throughout the test. The researcher that gave verbal encouragement was blinded to the IPC or CT exposure. Subjects had to achieve in the first sprint of the first set at least 95% of their BT obtained in the familiarization visit. Importantly, RSE has been shown to be reproducible and valid for assessing repeated sprint ability in team sports athletes (Rampinini et al., 2007; Impellizzeri et al., 2008). In addition, the protocol with multiple sets of RSE was chosen because it may resemble more accurately what occurs during team sports games (Serpiello et al., 2011).

Measurements

HR was recorded beat by beat (S810i, Polar, Finland) and pulmonary gas exchange breath by breath (K4b2, Cosmed, Italy) throughout the RSE task (Hausswirth et al., 1997; Gamelin et al., 2006; Vanderlei et al., 2008; Weippert et al., 2010). However, due to technical problems we did not record long-term HRR data from one subject and $\dot{V}O_2$ data from three

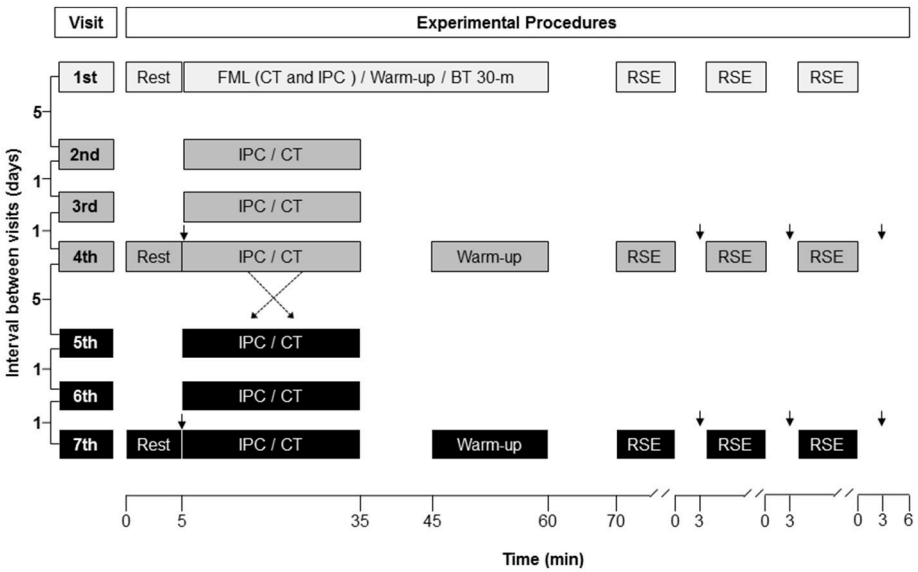

FIGURE 1 | Illustration of the experimental design. The protocol was randomized, controlled, crossed-over and double-blinded. FML, familiarization; CT, control procedure; IPC, ischemic preconditioning; BT 30-m, best time in a 30-m sprint, with a change of direction of 180° at the middle of the course (15 + 15 m); RSE, repeated sprint exercise; ↓, blood sample for blood lactate analysis.

subjects. Before each test, O_2 and CO_2 analyzers were calibrated according to the manufacturer's specifications using ambient air and gases with known concentration (16% O_2 and 4% CO_2). The flowmeter was calibrated using a 3-L syringe. An ointment was used in an ear lobe to induce vasodilation at pre-interventions and pre-RSE (Finalgon, Boehringer Mannheim, Germany). Then, a capillary blood sample (25 μL) was collected from the earlobe in a heparinized and calibrated capillary before the exposure to IPC and CT, as well as at 180 s of recovery after each RSE set. Each blood sample was stored in an Eppendorf containing 50 μL of 1% NaF (i.e., anticoagulant) and frozen at −20°C until analysis of blood lactate concentration ([Lac-]) (YSI 1500 SPORT, Yellow Springs Instruments, USA). Time of each sprint was measured with an accuracy of 0.001 s via the photocell system (Test Speed 6.0, CEFISE, Brazil). A photocell was placed 50 cm above the ground. Subjects had to stay 30 cm behind the photocell to avoid a false start at sprint departure.

Data Analysis

R-R intervals were extracted from the heart rate monitor and placed in a customized Excel spreadsheet. The difference between consecutive R-R intervals varied from 0 to 20% for 99% of all R-R intervals recorded in the study (29,636 R-R intervals). As we assessed young healthy mean, abnormal data most probably represented measurement artifacts due to bad contact between the thorax strip and the underling skin, rather than extra-systoles. Abnormal data were objectively identified by an automatic filter that highlighted any R-R interval differing more than 20% from the previous one. This procedure should preserve the physiological variability between successive R-R intervals, while removing artifacts and unlikely extra-systoles (Task Force, 1996). Abnormal data were then deleted and replaced by linear interpolation of adjacent data.

HRR from exercise shows a biphasic pattern consisting on a fast HR decay, which lasts about 60 s (Peçanha et al., 2014), followed by a slow HR decay, which is usually analyzed up to 360 s of recovery (Peçanha et al., 2014). Therefore, HR data recorded during the first 60 s of recovery from sets 1, 2 and 3 were used to calculate the following short-term indexes of the cardiac autonomic control (Buchheit et al., 2007; Nakamura et al., 2009; Peçanha et al., 2014): (1) negative reciprocal of the slope obtained from a linear regression between natural log-transformed HR and time using data from the first 30 s of recovery (T30); (2) absolute difference between 5-s mean HR at the end of a set (HRpeak) and 60 s later (HRR60s). HR data recorded until 360 s of recovery after set 3 were used to calculate a time constant (Tau) of a first-order exponential decay regression. HRR Tau therefore represented a long-term index of the cardiac autonomic control (Buchheit et al., 2007; Nakamura et al., 2009; Peçanha et al., 2014). In addition, RMSSD of 30 s data segments (i.e., RMSSD) provided an index of HRV from the onset to the end of set 3 recovery (Buchheit et al., 2007; Nakamura et al., 2009; Peçanha et al., 2014). T30, HRR60s and RMSSD were calculated in a customized Excel spreadsheet. Tau was calculated in the Origin 6.0 software (Microcal, USA). T30, HRR60s, Tau and RMSSD have shown to be valid indexes of cardiac autonomic control at post-exercise via pharmacological blockade studies (Imai et al., 1994; Goldberger et al., 2006). The reported coefficient of variation for these indexes after high-intensity intermittent exercise is: T30 = 73% (Dupuy et al., 2012), HRR60s = 11% (Bonato et al., 2018), Tau = 14% (Bonato et al., 2018) and RMSSD = 15–28% (Al Haddad et al., 2011). The investigator that analyzed all the data (T.R.L.) was not blinded to the conditions. However, raw R-R

intervals were objectively processed. Then, data were used for calculations using fixed mathematical parameters. Therefore, no step in the analysis process of R-R intervals was vulnerable to subjective data handling, and the same applies to the breath data analysis described next.

Breathing data were filtered to exclude aberrant breaths (two standard deviations from the mean of a 30-breath window) (Poole and Jones, 2012). Valid breath by breath values were linearly interpolated to get one value per second (Origin 6.0, Microcal, USA). Then, 5-s means were calculated. Peak oxygen uptake ($\dot{V}O_2$peak) and carbon dioxide output ($\dot{V}CO_2$peak) were taken as: (1) the highest 5-s mean of the last sprint of each set, and (2) the mean of the three highest 5-s values of each set. The two analyses provided similar results' interpretation, and so only the results from the former were presented. Peak respiratory exchange ratio (RERpeak) was calculated dividing $\dot{V}CO_2$peak by $\dot{V}O_2$peak. Kinetics of long-term $\dot{V}O_2$ recovery was represented by the Tau of a first-order exponential decay regression using $\dot{V}O_2$ data from the end of set 3 to the end of the subsequent 360-s recovery period (Rossiter et al., 2002). $\dot{V}O_2$ recovery kinetics was measured because of its association with phosphocreatine recovery kinetics (Rossiter et al., 2002), which, in turn, is largely dependent on the oxidative metabolism (Piiper and Spiller, 1970). Accumulation of blood [Lac-] (Δ[Lac-]) was quantified via deltas of two consecutive measurements (i.e., set 1 minus baseline; set 2 minus set 1; and set 3 minus set 2). At last, the following parameters were obtained to assess RSE performance: BT, total time (TT) and percent sprint performance decrement (%DC). The %DC was determined as follows: (100*(TT/ BT*6))-100 (Fitzsimons et al., 1993). The reported coefficient of variation for the BT, TT and %DC is 1.3, 0.8, and 30.2%, respectively (Impellizzeri et al., 2008).

Statistical Analysis

Sample size was calculated taking into account a two-way repeated measures ANOVA with two interventions (IPC and CT) and three repeated measures (sets 1, 2, and 3). The main endpoint was the HRR60s, given that this is valid (Kannankeril et al., 2004), reproducible (Al Haddad et al., 2011; Dupuy et al., 2012; Bonato et al., 2018) and widely used in clinical and sports settings to assess the post-exercise cardiac autonomic control (Buchheit, 2014; Peçanha et al., 2014). High-intensity interval training protocols provoked significant HRR60s increase ($P < 0.05$) at *d* effect size of 0.75 (Lamberts et al., 2009) and 0.87 (Villelabeitia-Jaureguizar et al., 2017). Both effects corresponded to an absolute increase of 6 bpm. We reasoned the IPC effect on HRR60s could be approximately half of such effects (d∼0.40; absolute delta ∼ 3 bpm). Next, the estimated *d* effect size had to be converted to a partial eta square (η^2_p) effect size to input an estimated effect in a repeated measures ANOVA. This resulted in a η^2_p of 0.04. Correlation among repeated measures was at 0.85 and nonsphericity correction at 1.0. Using these parameters, 14 subjects would be necessary to find a *P*-value lower than 0.05, with 0.80 of power (G*Power 3.1, Dusseldorf University, Germany).

Data distribution was verified by the Shapiro-Wilk's test. RMSSD did not present normal distribution, and so, was transformed to natural logarithm for inferential analyses. Paired Student's *t*-test was used to analyze HRR Tau, baseline [Lac-] and $\dot{V}O_2$ Tau. Two-way repeated measures ANOVA (factors: condition and set) was used to analyze HRpeak, short-term HRR, $\dot{V}O_2$peak, $\dot{V}CO_2$peak, RERpeak, [Lac-], Δ[Lac-], and RSE performance. Two-way repeated measures ANOVA (factors: condition and time) was used to analyze 5-s mean HR and RMSSD along 360 s after set 3. Three-way repeated measures ANOVA (factors: condition, set and time) was used to analyze 5-s mean HR throughout 60-s recovery periods after all sets. The Greenhouse-Geisser's correction was used to adjust ANOVA results, whenever sphericity was violated in the Mauchly's test. The LSD *post hoc* was used when significant F values were found. Effect sizes for Student's *t*-test and ANOVA results were calculated as Cohen's *d* and η^2_p, respectively. The following thresholds were used for *d* and η^2_p interpretation (Cohen, 1988): *d,* trivial > 0.00; small > 0.20; medium > 0.50; and large > 0.8/η^2_p, trivial > 0.00; small > 0.01; medium > 0.06; large > 0.14. Results are presented as mean ± standard error of mean (SEM). Statistical significance was set at $P < 0.05$. Statistical analyses were performed in the software Statistica 12 (Statsoft, EUA).

RESULTS

HR from peak to 25 s of recovery was similar between IPC and CT in all sets (**Table 1** and **Figure 2**), leading to similar T30 (**Table 1**). On the other hand, HR from 30 to 60 s of recovery was lower (medium η^2_p) in the IPC than CT in all sets (**Figure 2**), and, consequently, IPC increased HRR60s (**Table 1**; large η^2_p; change = 12.8%, 95% CI = 5.8–19.7%). HR values were similar between conditions throughout 360 s of recovery from set 3 (**Figure 3**), resulting in similar HRR Tau (**Table 1**). RMSSD was also similar between IPC and CT throughout the entire recovery from set 3 (**Figure 4**). No difference between IPC and CT was observed for baseline [Lac-] (IPC: 1.30 ± 0.16 mmol.L^{-1} and CT: 1.24 ± 0.15 mmol.L^{-1}; $P = 0.45$; $d = 0.20$), as well as for $\dot{V}O_2$peak, $\dot{V}CO_2$peak, RERpeak, [Lac-] and Δ[Lac-] and $\dot{V}O_2$ Tau (**Table 2**). Additionally, RSE performance was similar between conditions (**Table 3**).

DISCUSSION

Our main finding was that IPC increased HRR60s which, in addition to being a valid (Kannankeril et al., 2004) and reproducible parameter (Al Haddad et al., 2011; Dupuy et al., 2012; Bonato et al., 2018), is the most used method to assess the post-exercise cardiac autonomic control in clinical and sports settings (Buchheit, 2014; Peçanha et al., 2014). This result is novel and may therefore have practical implications. However, contrary to our hypothesis, the IPC-mediated cardiac autonomic control improvement was not accompanied by changes in $\dot{V}O_2$, $\dot{V}CO_2$, RER, and blood [Lac-]. Thus, collectively, the results indicate that IPC improved an important index of the short-term recovery of cardiac autonomic control from RSE, regardless of change in surrogates of energy metabolism responses to RSE.

TABLE 1 | Peak heart rate (HRpeak), short-term and long-term heart rate recovery (HRR) after each set of repeated sprint.

					ANOVA *P*-value (η^2_p)			Student's *t*-test *P*-value (*d*)
		SET 1	SET 2	SET 3	Condition	Set	Interaction	
HRpeak (bpm)	CT	181 ± 2	183 ± 2	185 ± 2	0.76 (0.01)	0.01 (0.33)	0.14 (0.14)	NA
	IPC	182 ± 2	183 ± 2	185 ± 2				
T30 (s)	CT	435 ± 81	434 ± 55	407 ± 48	0.15 (0.16)	0.42 (0.07)	0.48 (0.04)	NA
	IPC	365 ± 56	427 ± 65	374 ± 47				
HRR60s (bpm)	CT	34 ± 3	33 ± 2	34 ± 3	0.03 (0.27)	0.24 (0.09)	0.69 (0.03)	NA
	IPC	39 ± 3	36 ± 3	38 ± 3				
HRR Tau (s)	CT	NA	NA	78 ± 4	NA	NA	NA	0.64 (−0.14)
	IPC			78 ± 6				

Data are mean ± SEM. T30, negative reciprocal of the slope obtained from a linear regression between natural log-transformed heart rate (HR) and time using data from the first 30 s of recovery; HRR60s, absolute difference between 5-s mean HR at the end of a set and 60 s later; Tau, time constant of a first-order exponential decay regression after set 3; CT, control; IPC, ischemic preconditioning; η^2_p, partial eta square; NA, not applicable; HRpeak, T30 and HRR60s n = 15; HRR Tau n = 14.

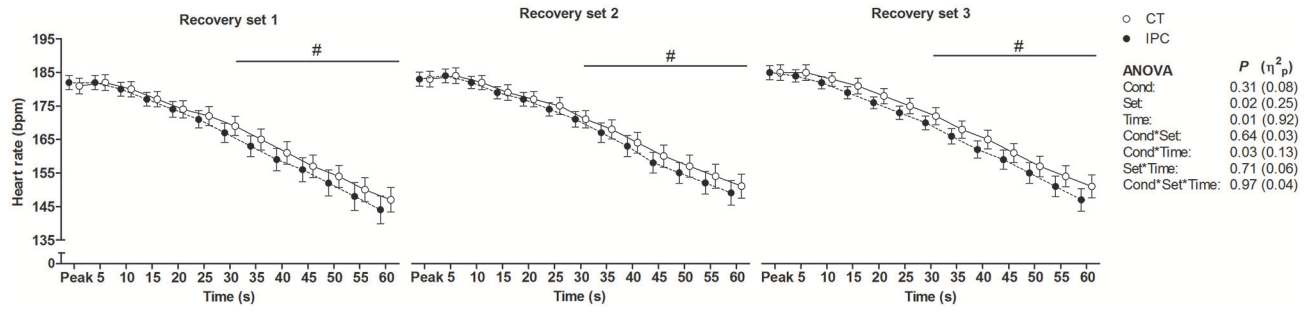

FIGURE 2 | Short-term component of heart rate recovery after each set of repeated sprint exercise. Data were shifted in the X axis to avoid overlapping and thus improve visualization. Data are mean ± SEM. $n = 15$. $^{\#}P < 0.05$ between conditions in the *post hoc* analysis of Cond * Time interaction. CT, control; IPC, ischemic preconditioning; Cond, condition; η^2_p, partial eta square.

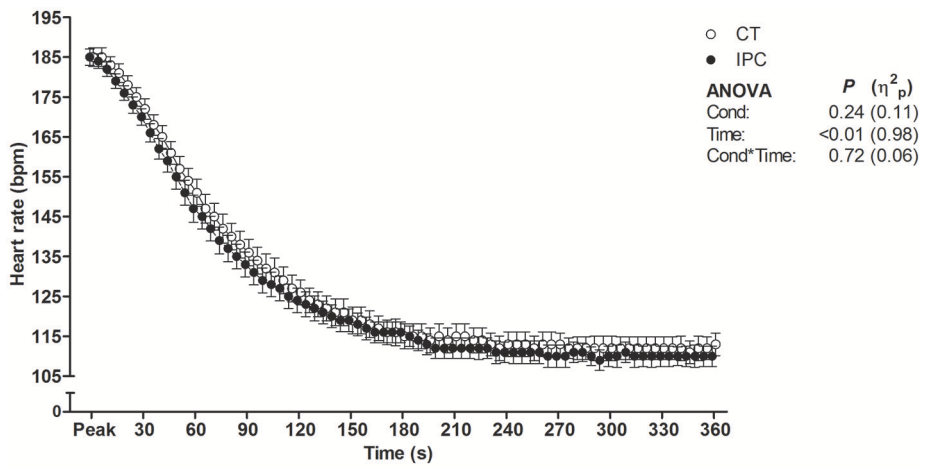

FIGURE 3 | Heart rate throughout 360 s of recovery after set 3. Data were shifted in the X axis to avoid overlapping and thus improve visualization. Data are mean ± SEM. $n = 14$. CT, control; IPC, ischemic preconditioning; Cond, condition; η^2_p, partial eta square.

Effect of IPC on Post-exercise Cardiac Autonomic Control

In our study IPC did not change HRpeak. This finding is similar to those reported by studies that investigated the IPC effect on HRpeak during high-intensity intermittent exercise (Marocolo et al., 2017; Zinner et al., 2017) or HRpeak during ramp incremental exercise (de Groot et al., 2010; Crisafulli et al., 2011; Sabino-Carvalho et al., 2017). As far as we know, only two studies

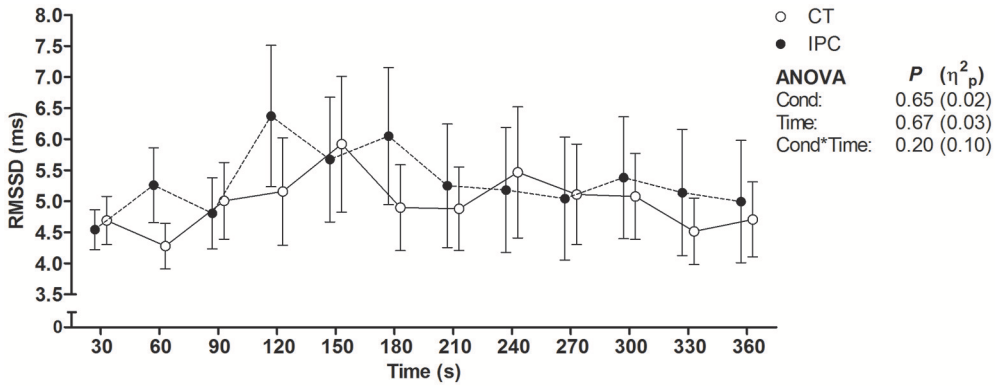

FIGURE 4 | Root mean square of successive differences between R-R intervals (RMSSD) throughout 360 s of recovery after set 3. Data were shifted in the X axis to avoid overlapping and thus improve visualization. Data are mean ± SEM. $n = 14$. CT, control; IPC, ischemic preconditioning; Cond, condition; η^2_p, partial eta square.

TABLE 2 | Energy metabolism responses to repeated sprint exercise.

					ANOVA *P*-value (η^2_p)		
		SET 1	**SET 2**	**SET 3**	**Condition**	**Set**	**Interaction**
$\dot{V}O_2$peak (mL.kg^{-1}.min^{-1})	CT	38.45 ± 1.84	37.68 ± 1.64	36.93 ± 1.93	0.95 (0.00)	0.14 (0.17)	0.98 (0.00)
	IPC	38.25 ± 1.74	37.58 ± 1.51	37.02 ± 1.86			
$\dot{V}CO_2$peak (mL.kg^{-1}.min^{-1})	CT	49.08 ± 1.71	44.32 ± 0.88	42.50 ± 1.42	0.80 (0.01)	<0.01 (0.63)	0.97 (0.00)
	IPC	49.01 ± 1.28	43.96 ± 1.16	42.00 ± 1.66			
RER	CT	1.30 ± 0.05	1.20 ± 0.05	1.18 ± 0.07	0.78 (0.01)	<0.01 (0.63)	0.73 (0.03)
	IPC	1.30 ± 0.05	1.19 ± 0.05	1.15 ± 0.05			
[Lac-] (mmol.L^{-1})	CT	11.05 ± 0.61	14.50 ± 0.96	14.54 ± 1.09	0.81 (0.00)	<0.01 (0.70)	0.84 (0.01)
	IPC	11.06 ± 0.82	14.12 ± 1.11	14.30 ± 1.06			
Δ[Lac-] (mmol.L^{-1})	CT	9.82 ± 0.67	3.44 ± 0.62	0.04 ± 0.41	0.73 (0.01)	<0.01 (0.89)	0.89 (0.01)
	IPC	9.76 ± 0.80	3.06 ± 0.52	0.18 ± 0.61			

Data are mean ± SEM. $\dot{V}O_2$peak, peak oxygen uptake; $\dot{V}CO_2$peak, peak carbon dioxide output; RERpeak, peak respiratory exchange ratio; [Lac-], blood lactate concentration; Δ[Lac-], difference between consecutive blood lactate measures; CT, control; IPC, ischemic preconditioning; η^2_p,partial eta square. $\dot{V}O_2$peak, $\dot{V}CO_2$peak and RERpeak n = 12. [Lac-] and Δ[Lac-] n = 15.

have reported the IPC effect on post-exercise cardiovascular parameters. Both studies used handgrip exercise and employed circulatory occlusion during recovery from exercise to isolate the activation of the muscle metaboreflex. Incognito et al. (2017) reported IPC did not change arterial pressure, HR and muscle sympathetic nerve activity during exercise and post-exercise circulatory occlusion. Mulliri et al. (2016) reported IPC did not change central and peripheral hemodynamic parameters during exercise, but IPC decreased mean arterial pressure during post-exercise circulatory occlusion due to a venous return-induced reduction of stroke volume and cardiac output. Of note, however, Incognito et al. (2017) and Mulliri et al. (2016) did not provide enough data to interpret the IPC effect under normal free flow recovery from exercise, like we did in the present study. In addition, cardiovascular responses to handgrip exercise are very different from those provoked by dynamic exercise involving large muscle mass (Lewis et al., 1985). For example, Incognito et al. (2017) and Mulliri et al. (2016) reported mean HR to achieve 102 and 72 bpm, respectively. In contrast, in our study HR surpassed 180 bpm. Therefore, methodological dissimilarities preclude comparison of the IPC effect on post-exercise cardiovascular parameters between former studies (Mulliri et al., 2016; Incognito et al., 2017) and the present one.

We found that IPC did not change HRR up to 25 s, T30, HRR Tau and RMSSD. Conversely, IPC consistently lowered HR from 30 to 60 s, leading to increased HRR60s after all sets. Thus, our results indicate that IPC only had an effect on the second half of the short-term HRR. A possible explanation for the divergent IPC effect on short-term HRR indexes is that the RSE deleterious effect on HRR was so powerful that HR did not decay within approximately the first 10 s of recovery (i.e., onset HRR). As a result, the T30 index which relies on onset recovery data may have not been sensitive to assess the short-term component of post-RSE HRR. This phenomenon may dependent on the exercise intensity, given that it has also been reported by other studies that assessed post-RSE HRR (Buchheit et al., 2007; Nakamura et al., 2009; Del Rosso et al., 2017), but not by studies that assessed HRR after submaximal or maximal incremental exercise in healthy subjects (Imai et al., 1994; Kannankeril et al., 2004). Another point is that the IPC effect in the present study

TABLE 3 | Repeated sprint exercise performance.

					ANOVA *P*-value (η^2_p)		
		SET 1	**SET 2**	**SET 3**	**Condition**	**Set**	**Interaction**
BT (s)	CT	5.9 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	0.23 (0.10)	<0.01 (0.55)	0.46 (0.05)
	IPC	5.8 ± 0.1	6.0 ± 0.2	6.1 ± 0.1			
TT (s)	CT	37.2 ± 0.4	38.6 ± 0.4	39.4 ± 0.6	0.09 (0.19)	<0.01 (0.70)	0.81 (0.01)
	IPC	36.5 ± 0.3	37.9 ± 0.4	38.6 ± 0.5			
%DC	CT	4.4 ± 0.4	6.7 ± 0.6	6.7 ± 0.8	0.07 (0.21)	<0.01 (0.40)	0.10 (0.23)
	IPC	4.3 ± 0.4	5.3 ± 0.5	5.8 ± 0.6			

Data are mean ± SEM. BT, best time; TT, total time; %DC, percent sprint performance decrement; CT, control; IPC, ischemic preconditioning; η^2_p, partial eta square; n = 15 for all variables.

may have not been large enough to overcome the variability of parameters with low-to-moderate reproducibility, such as T30 and RMSSD. On the other hand, the absence of IPC effect on the HRR Tau was unequivocal, due to equal mean values between IPC and CT, high Student's *t*-test *P*-value and reasonable reproducibility of this parameter (Bonato et al., 2018). These facts therefore clearly support that IPC did not change long-term post-RSE HRR.

Previous studies have used systemic pharmacological blockade of vagal (i.e., muscarinic) and sympathetic (i.e., beta) receptors aiming to dissect the contribution of each branch of the autonomic nervous system to HRR (Imai et al., 1994; Kannankeril et al., 2004; Goldberger et al., 2006). These studies showed both short- and long-term HRR are largely determined by cardiac vagal reactivation post-submaximal exercise, with a negligible influence of the sympathetic branch. However, no study has investigated the autonomic contribution to HRR after supramaximal exercise (i.e., exercise performed at velocity or workload higher than peak velocity or workload of an incremental exercise test), such as RSE, which may not be the same as post-maximal exercise. Firstly, because our study and others showed RMSSD almost did not recover post-RSE (Buchheit et al., 2007; Nakamura et al., 2009; Del Rosso et al., 2017), which is not the case post-maximal exercise (Goldberger et al., 2006). Secondly, because the level of circulating catecholamines follows exercise-induced lactic acidosis (Mazzeo and Marshall, 1989) and RSE usually leads to greater lactic acidosis than maximal exercise (Buchheit et al., 2007; Sabino-Carvalho et al., 2017). Thirdly, because intense muscle metaboreflex activation during recovery from exercise under circulatory occlusion increases cardiac sympathoexcitation and offsets the influence of cardiac vagal activation on HR (Fisher et al., 2010). In sum, although cardiac vagal reactivation possibly plays a crucial role for short-term post-RSE HRR, sympathoexcitation is greater during RSE than maximal exercise. Consequently, cardiac sympathetic activity perhaps restrains to some extent short-term post-RSE HRR, and cardiac sympathetic withdrawal may importantly contribute to long-term post-RSE HRR. Thus, in our study, IPC-induced acceleration of HRR60s was possibly mediated by greater cardiac vagal reactivation, but lower cardiac sympathetic restraint cannot be disregarded. In contrast, as IPC did not change HRR after 60 s of recovery and RMSSD along 360 s of recovery, cardiac vagal reactivation and, particularly, cardiac sympathetic withdrawal likely did not change during the long-term component of cardiac autonomic control recovery.

Effect of IPC on Energy Metabolism Responses

IPC increased peak pulmonary oxygen uptake ($\dot{V}O_2$) in a sample mostly composed by men (de Groot et al., 2010; Cruz et al., 2015) and decreased blood lactate concentration [Lac-] in men (Bailey et al., 2012) during incremental dynamic exercise. In contrast, Patterson et al. (2015) showed no effect of IPC on $\dot{V}O_2$, $\dot{V}CO_2$, and blood [Lac-] during twelve 6-s cycling sprints in men. Gibson et al. (2015) reported IPC to reduce blood [Lac-] in women, but not men, post five 6-s cycling sprints. More recently, Griffin et al. (2018) did not find an effect of IPC on blood [Lac-] during three sets of six shuttle run sprints in men. The reason for the dissimilar IPC effect on surrogates of energy metabolism during incremental dynamic exercise vs. RSE is still unclear. One possibility could be that the IPC dose has been insufficient for RSE. Thus, herein we employed a repeated IPC protocol. Nevertheless, we also found that IPC changed none of the assessed surrogates of energy metabolism. This result consequently suggests that the IPC dose may not be an issue. Possible modulators of the IPC effect, such as gender (Gibson et al., 2015), physical fitness (Sabino-Carvalho et al., 2017), and time between IPC application and exercise assessments (Lisbôa et al., 2017) should then be taken into consideration by next studies. Still of note, our study and others (de Groot et al., 2010; Crisafulli et al., 2011; Bailey et al., 2012; Cruz et al., 2015; Gibson et al., 2015; Patterson et al., 2015; Griffin et al., 2018) have measured $\dot{V}O_2$ and $\dot{V}CO_2$ via analysis of breathing air and [Lac-] via analysis of capillary blood, but these methods only provide indirect information with regards to muscle aerobic and anaerobic responses. Thus, other methods should be employed in the future to confirm the IPC effect on energy metabolism responses to RSE.

As IPC did not change energy metabolism surrogates during a RSE task, the acceleration of HRR60s was possibly mediated by other factors than energy metabolism responses to RSE. In this sense, IPC has been linked with release of humoral factors by preconditioned tissues (e.g., adenosine, bradykinin, and

calcitonin), as well as by activation of afferent neural pathways in preconditioned tissues (e.g., C- and Aδ-fibers) (Gourine and Gourine, 2014). Humoral and neural mechanisms have thus been considered triggers of IPC-induced vagal activation during ischemia-reperfusion injury protocols (Gourine and Gourine, 2014). Therefore, direct vagal activation via neural and humoral mechanisms likely underlies the IPC effect on the HRR60s, rather than an indirect IPC effect on energy metabolism responses to exercise.

Some studies have shown that IPC can enhance exercise performance due to a placebo effect rather than a specific IPC effect (Marocolo et al., 2015, 2016; Sabino-Carvalho et al., 2017). Noteworthy, the IPC placebo effect on exercise performance was not accompanied by change in energy metabolism and HR responses to exercise (Marocolo et al., 2015, 2016; Sabino-Carvalho et al., 2017). Our experimental design did not allow dissecting specific IPC effects from placebo effects. However, based on the aforementioned studies (Clark et al., 2000; Foad et al., 2008; Marocolo et al., 2015, 2016; Sabino-Carvalho et al., 2017), it is possible that sprints time were more prone to placebo and nocebo effects than physiological measurements.

Implications

HRR60s percent change (12.8% for IPC main effect) surpassed the smallest worthwhile change (6%, calculated as 0.2 × between-subjects standard deviation for all sets in the CT condition) and the coefficient of variation elsewhere reported (i.e., 11%) (Bonato et al., 2018). In addition, the HRR60s absolute change in the present study (i.e., 4 bpm) was somewhat comparable to the absolute change provoked by 8 weeks of high-intensity interval training in patients with coronary disease (i.e., 6 bpm) (Villelabeitia-Jaureguizar et al., 2017) and by 3 weeks of high-intensity interval training in well-trained cyclists (i.e., 5 bpm) (Lamberts et al., 2009). The IPC effect herein observed therefore achieved a magnitude that could carry practical implications for clinical populations and athletes which deserves investigation by next studies. With regard to clinical populations, studies in dogs strongly support that the higher the HRR and the cardiac vagal outflow to the heart, the lower is the chance of developing ventricular fibrillation and dyeing after induction of acute myocardial ischemia during exercise (Vanoli et al., 1991; Smith et al., 2005). Although we assessed healthy young men, our results suggest IPC could carry cardioprotective benefits for subjects with cardiovascular risk factors or stablished cardiovascular diseases that engage in RSE training or practice of sports that may involve RSE, such as soccer, basketball, tennis, etc. With regard to athletes, a better post-exercise cardiac autonomic control may indicate greater readiness to train at high-intensity (Borresen and Lambert, 2007; Kiviniemi et al., 2010; Buchheit, 2014; Capostagno et al., 2014). Thus, the administration of IPC before high-intensity interval training sessions could enhance athletes' training tolerance, resulting in amplified or accelerated generation of chronic adaptations.

CONCLUSION

IPC accelerated to some extent the short-term recovery, but did not change the long-term recovery of cardiac autonomic control from RSE, and such accelerator effect was not accompanied by any IPC effect on surrogates of energy metabolism responses to RSE.

DATASETS AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

TL, JS, AS, and BS conception and design of the study. TL, JS-C, TF, and BS data acquisition. TL and BS data analysis and interpretation. TL and BS wrote the manuscript. TL, JS-C, TF, JS, AS, and BS critical review of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Enhanced Metabolic Stress Augments Ischemic Preconditioning for Exercise Performance

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Purpose: To identify the combined effect of increasing tissue level oxygen consumption and metabolite accumulation on the ergogenic efficacy of ischemic preconditioning (IPC) during both maximal aerobic and maximal anaerobic exercise.

Methods: Twelve healthy males (22 ± 2 years, 179 ± 2 cm, 80 ± 10 kg, 48 ± 4 ml·kg^{-1}·min^{-1}) underwent four experimental conditions: (i) no IPC control, (ii) traditional IPC, (iii) IPC with EMS, and (iv) IPC with treadmill walking. IPC involved bilateral leg occlusion at 220 mmHg for 5 min, repeated three times, separated by 5 min of reperfusion. Within 10 min following the IPC procedures, a 30 s Wingate test and subsequent (after 25 min rest) incremental maximal aerobic test were performed on a cycle ergometer.

Results: There was no statistical difference in anaerobic peak power between the no IPC control (1211 ± 290 W), traditional IPC (1209 ± 300 W), IPC + EMS (1206 ± 311 W), and IPC + Walk (1220 ± 288 W; $P = 0.7$); nor did VO_2max change between no IPC control (48 ± 2 ml.kg^{-1}.min^{-1}), traditional IPC (48 ± 6 ml.kg^{-1}.min^{-1}), IPC + EMS (49 ± 4 ml.kg^{-1}.min^{-1}) and IPC + Walk (48 ± 6 ml.kg^{-1}.min^{-1}; $P = 0.3$). However, the maximal watts during the VO_2max increased when IPC was combined with both EMS (304 ± 38 W) and walking (308 ± 40 W) compared to traditional IPC (296 ± 39 W) and no IPC control (293 ± 48 W; $P = 0.02$).

Conclusion: This study shows that in a group of participants for whom a traditional IPC stimulus was not effective, the magnification of the IPC stress through muscle contractions while under occlusion led to a subsequent exercise performance response. These findings support that amplification of the ischemic preconditioning stimulus augments the effect for exercise capacity.

Keywords: exercise, hypoxia, occlusion, cycling, metabolites

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INTRODUCTION

It has been demonstrated that brief periods of circulatory occlusion and reperfusion, or ischemic preconditioning (IPC), can act to improve exercise performance (Jean-St-Michel et al., 2011; Bailey et al., 2012). Multiple studies have demonstrated that IPC performed in the minutes to hours preceding aerobic (De Groot et al., 2010) or anaerobic (Patterson et al., 2015; Cruz et al., 2016)

exercise can improve performance but there appears to be great variability in response and, at present, the magnitude and consistency of the IPC effect across populations is not clear for either aerobic (Bailey et al., 2012; Hittinger et al., 2014; Sabino-Carvalho et al., 2017) nor anaerobic (Lalonde and Curnier, 2014; Paixao et al., 2014) exercise. Contributing to the lack of clarity around IPC as an effective ergogenic aid is the fact that the physiological signaling stimuli and associated downstream responses remain incompletely characterized. Of the leading physiological theories, local hypoxia [leading to HIF-1 signaling (Eckle et al., 2008)] and metabolite accumulation [such as adenosine, bradykinin, ROS, and opioids (Cohen et al., 2000; Marongiu and Crisafulli, 2014)] have received considerable attention; however, the existence of a dose-response relationship or identification of a threshold to trigger the biochemical pathways leading to the IPC effect remain unconfirmed (Cohen et al., 2000; Marongiu and Crisafulli, 2014). Given the many variations of IPC methodology reported in the current literature (i.e., differences in duration and number of cycles, occlusion pressure, volume of restricted muscle mass, local exercising, or remote muscle group) defining a pattern of the most efficacious method remains a challenge.

The metaboreflex is a key factor in controlling sympathetic outflow during exercise (Alam and Smirk, 1937) and studies utilizing ischemia to amplify metabolites and provide increased afferent feedback have shown an elevated sympathetic outflow and blood pressure response (Rowell et al., 1991; Tschakovsky and Hughson, 1999). Provided that the accumulation of metabolites is adequate, IPC could promote metaboreflex-induced increases in sympathetic outflow and blood pressure, preparing the body for subsequent exercise. IPC alone, however, has not been shown to elicit a sympathetic response, whereas the combination of cyclic bouts of blood flow restriction-reperfusion and treadmill exercise at 65% heart rate max has (Sprick and Rickards, 2017). It remains unclear if this combination can lead to improvements in performance, but it is possible that a sufficient metabolic stimulus (intramuscular perturbation) of IPC may be a crucial factor to elicit the desired effect.

By combining IPC with light exercise, such as walking, the muscle contractions thus evoked could function to amplify the hypoxic and/or metabolic preconditioning stimulus. As exercising while under blood flow occlusion may not be feasible or practical in certain situations (e.g., limited mobility during travel or when other temporal or spatial limitations exist in warm-up), the passive technique of electrical muscle stimulation (EMS) may be a more suitable option to similarly combine muscle contractions with IPC. Thus, we were interested in attempting to amplify the IPC performance effect by combining IPC with either active walking or passive EMS to enhance the stimulus evoked during a single treatment session. Both the active and passive models represent possible pre-competition strategies to increase tissue level hypoxia and metabolite accumulation compared with IPC alone.

Ischemic preconditioning is most commonly performed using supra-arterial occlusion pressures, dictating that both arterial inflow and venous outflow are subsequently restricted. As such there is a direct, and perhaps unavoidable, link between a greater emphasis on anaerobic metabolism and metabolite accumulation under these conditions which is challenging to meaningfully disentangle (Scott et al., 2014). Therefore, the purpose of this study was to identify the combined effect of increasing tissue level oxygen consumption and subsequent metabolite accumulation on the ergogenic efficacy of IPC during both maximal aerobic and maximal anaerobic exercise. It was hypothesized that IPC combined with muscle contractions induced by slow walking or electrical muscle stimulation would augment the ergogenic IPC effect, as demonstrated by greater aerobic and anaerobic power outputs.

MATERIALS AND METHODS

Subjects

Twelve healthy males (22 ± 2 years, 179 ± 2 cm, 80 ± 10 kg, 47.7 ± 4 $ml \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in this study which employed a randomized cross-over design. All participants were recreationally active non-smokers. Participants had no medical history of chronic disease and were safe to exercise as confirmed through completion of a $PARQ^{+}$ screening questionnaire (Warburton et al., 2014). This study was carried out in accordance with the recommendations of the University of Guelph's human ethics research board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Guelph's human ethics research board (REB# 15SE019).

Protocol and Measurements

All participants refrained from alcohol, caffeine, and intensive physical exercise for a least 24 h prior to testing. On each of four visits to the lab, participants performed both a 30 s anaerobic Wingate test with a standardized 5-min warm-up and warm-down and, after a 25-min rest, a subsequent incremental maximal exercise test. Both tests were completed on a cycle ergometer (Velotron Inc., Seattle, WA, United States). The four experimental visits were performed at least 1 week apart and at the same time of day. Each visit involved either (i) baseline control involving no IPC, (ii) traditional IPC, (iii) IPC in combination with EMS, and (iv) IPC in combination with treadmill walking (2 mph at 0% grade). Ten minutes following the IPC procedures (described below), the performance tests were initiated as per the graphical representation of the protocol presented in **Figure 1**. To eliminate possible training, learning, or familiarization effects, all conditions were assigned in a random order. Participants, who otherwise had little in the way of expectations concerning the expected effects, were blinded to all performance data and were not informed *a priori* as to the expected outcomes of the study to avoid introducing possible placebo or nocebo effects.

Ischemic Preconditioning

Ischemic preconditioning was performed prior to exercise in a seated position using bilateral arterial occlusion. The occlusion

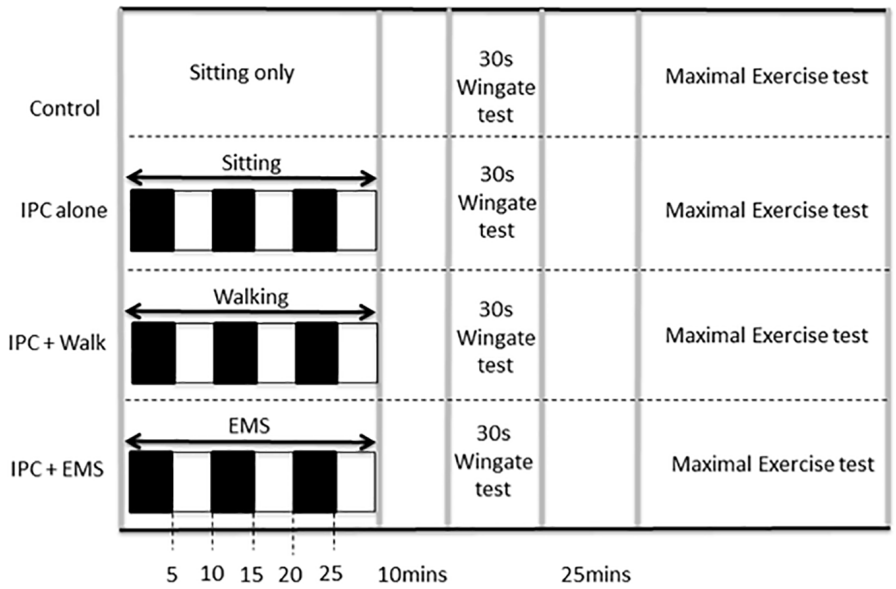

FIGURE 1 | Study protocol including experimental and control visits, which were performed in random order. *IPC* ischemic preconditioning, *EMS* electrical muscle stimulation. Black boxes represent arterial occlusion on both the right and left leg, white boxes represent no occlusion.

cuffs (Zimmer ATS 1500; United States) were positioned around the proximal thigh and inflated to 220 mmHg for 5 min. This procedure, which is most commonly used in the IPC exercise performance literature (Incognito et al., 2016), promotes complete occlusion of both the arterial inflow and venous outflow in the lower limbs throughout the 5 min (Kooijman et al., 2008) as was confirmed in the present study using a near-infrared spectroscopy (MOXY, MN, United States), and the disappearance of a distal pulse. This ischemic procedure was repeated three times, each separated by 5 min of reperfusion (Incognito et al., 2016). IPC in combination with EMS was also performed in a seated position and involved the above-mentioned IPC protocol with electrically evoked muscle contractions throughout. The EMS (Compex International, Mi-Runner Sport, United Kingdom) involved two surface electrodes placed on both the Vastus Medialis and Vastus Lateralis at the distal and proximal position that best elicited a muscular contraction. Stimulation was applied using a pulse train length of 400 μs, delivered at a frequency of 50–100 Hz at a maximally tolerable intensity level. As participants accommodated to the stimulation during a session, the stimulation intensity was progressively increased. IPC in combination with walking involved the above-mentioned IPC protocol with slow walking on a standard motor driven treadmill (Sole F63 treadmill, Canada) at 2 mph (Sakamaki et al., 2011).

30 s Anaerobic Wingate Test

The 30 s Anaerobic Wingate test included a "flying start," which consisted of 40 s of low load (100 W) pedaling prior to the introduction of the resistance (7.5% body weight), against which participants aimed to maintain maximal pedal revolutions for 30 s. Integrated Wingate testing software was used to calculate peak power output in watts.

Incremental Maximal Aerobic Capacity Test

The incremental exercise test began with a resistance of 100 W and increased continuously at 1 watt every 3 s until exhaustion (i.e., the participant was unable to maintain a pedaling frequency of ≥50 rpm). Starting 1-min prior, and continuing throughout the maximal exercise test, oxygen consumption (VO_2) was measured via indirect calorimetry using a face mask and optical turbine connected to a gas analyser with a sampling line (Cosmed Quark CPET, Rome, Italy). The maximal values were recorded as the highest reading that occurred after the data was smoothed using a rolling 30 s average. Attainment of true physiological max was confirmed for all subjects by the presentation of a plateau in VO_2 (increase in ≤50 mL/min at VO_2 peak and the closest neighboring data point), or respiratory exchange ratio (RER) ≥1.15 (Astorino et al., 2000). During the graded exercise test, VO_2 at submaximal intensities were recorded and compared every 20 W between 120 and 200 W to investigate possible effects on submaximal exercise efficiency.

Statistics

A Shapiro–Wilk test was used to confirm normality of data, prior to analysis. Comparisons between conditions were performed using repeated measures ANOVA, with LSD *post hoc* tests, as was appropriate. Statistical analyses were conducted using SPSS software (version 25; IBM, Chicago, IL, United States), with differences considered to be statistically significant at

$P < 0.05$. All data is presented as mean ± SD, unless specified otherwise.

RESULTS

30 s Anaerobic Wingate Test

Peak anaerobic power was 1211 ± 290 W during the no IPC control and 1209 ± 300 W following traditional IPC. When IPC was combined with EMS and walking, peak anaerobic power was recorded to be 1206 ± 311 W and 1220 ± 288 W, respectively. There were no statistical differences between any groups ($P = 0.7$).

Incremental Maximal Aerobic Capacity Test

Baseline VO_2max was 47.7 ± 4 ml·kg^{-1}.min^{-1} and 48.4 ± 6 ml·kg^{-1}·min^{-1} following traditional IPC. When IPC was combined with EMS and then walking, VO_2max was recorded to be 49.1 ± 4 ml·kg^{-1}·min^{-1} and 48 ± 6 ml·kg^{-1}·min^{-1}, respectively. There were no statistical differences between any groups ($P = 0.3$; **Figures 2A,B**). Submaximal oxygen consumption increased as the test progressed from 120 to 200 W, but these increases in VO_2 every 20 W were similar in their pattern and magnitude across all conditions (**Table 1**).

Peak watts, recorded at the point of exhaustion during the incremental maximal aerobic test, was 293 ± 48 W during the no IPC control and 296 ± 39 W following traditional IPC treatment. When IPC was combined with EMS and walking, peak watts increased to 304 ± 38 W and 308 ± 40 W, respectively (**Figures 3A,B**). Statistical analyses revealed significant increases in peak watts when combing IPC with EMS ($P = 0.02$) and walking ($P = 0.03$) compared to IPC alone. There were also significant increases in peak watts when combining IPC with EMS (0.04) and walking ($P = 0.002$) compared to the control group.

DISCUSSION

The present study sought to compare the effects of traditional IPC with an enhanced preconditioning stimulus, involving IPC combined with EMS or walking, for augmenting either aerobic and anaerobic performance. The main novel findings were that (1) IPC, when combined with walking or EMS significantly improved peak watt output in the maximal aerobic test to exhaustion, despite traditional IPC causing no significant benefit; (2) neither IPC nor an augmented adaptation of IPC improved maximal oxygen consumption; (3) neither IPC alone nor augmented IPC improved maximal anaerobic power. These findings suggest that a certain magnitude of metabolic and/or hypoxic stimulus may, thus, be important for stimulating the positive effects of IPC on exercise capacity, but that this effect was not driven by a change in aerobic or anaerobic maximal capacity.

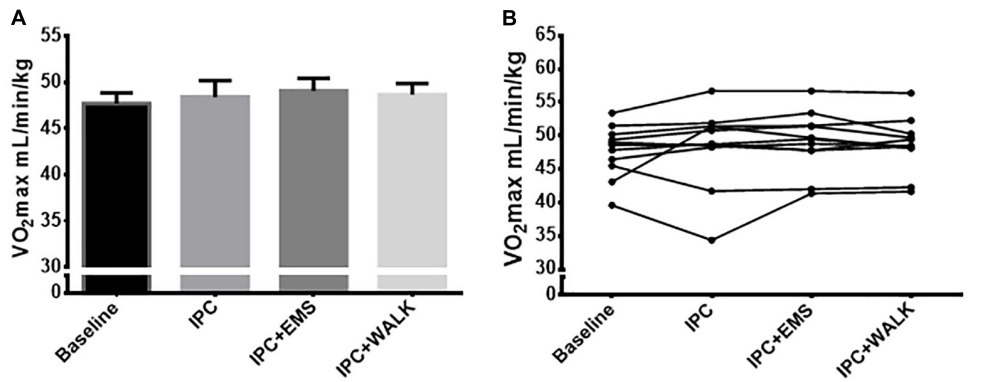

FIGURE 2 | (A) Mean maximal oxygen uptake ml.kg^{-1}.min^{-1} from the incremental maximal aerobic test for each intervention and baseline. **(B)** Maximal oxygen uptake ml.kg^{-1}.min^{-1} from the incremental maximal aerobic test for each intervention and baseline. Data is presented as mean ± SE.

TABLE 1 | Oxygen consumption at submaximal exercise intensities during an incremental cycling test after no intervention (Control) ischemic preconditioning (IPC), ischemic preconditioning combined with electrical muscle stimulation (IPC + EMS), and ischemic preconditioning performed during slow walking at 2 mph (IPC + Walk).

	Control	IPC	IPC + EMS	IPC + Walk	*P*-value
$VO_2$120 W (ml O_2·kg^{-1}·min^{-1})	25 ± 3	25 ± 4	25 ± 3	25 ± 2	0.9
VO_2 140 W (ml O_2·kg^{-1}·min^{-1})	28 ± 2	27 ± 4	28 ± 3	27 ± 3	0.5
VO_2 160 W (ml O_2·kg^{-1}·min^{-1})	30 ± 4	30 ± 4	30 ± 2	30 ± 3	0.8
VO_2 180 W (ml O_2·kg^{-1}·min^{-1})	33 ± 4	32 ± 4	33 ± 3	33 ± 3	0.5
VO_2 200 W (ml O_2·kg^{-1}·min^{-1})	35 ± 4	35 ± 4	36 ± 3	35 ± 3	0.8

Data is presented as mean ± SD.

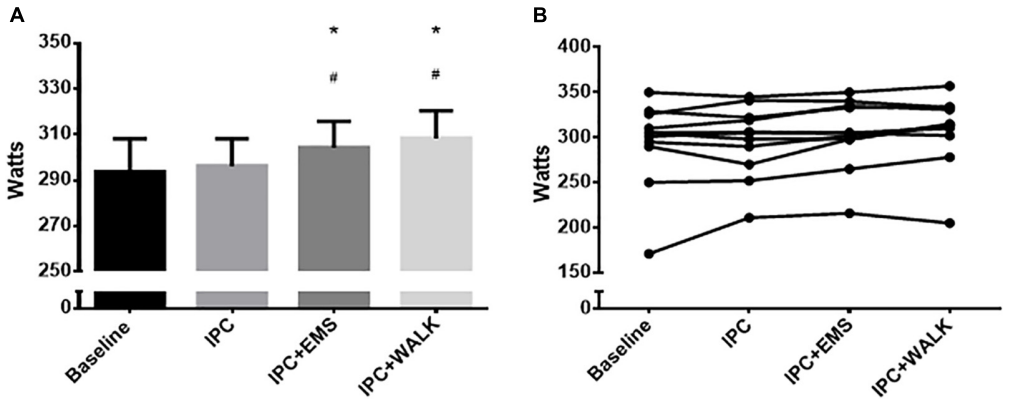

FIGURE 3 | (A) Mean peak watts from the incremental maximal aerobic test for each intervention and baseline. Data is presented as mean ± SE and the differences were considered significant at $P \leq 0.05$. *Represents statistically different from baseline; #Represents statistically different from IPC alone. **(B)** Individual peak watts from the incremental maximal aerobic test for each intervention and baseline.

Exercise Performance

Previous studies have demonstrated increases in cycling peak power output (of 1.6–3.7%) following IPC treatment during maximal tests (De Groot et al., 2010; Crisafulli et al., 2011). The current data demonstrate traditional IPC to be ineffective for increasing peak power output during a maximal cycling test; however, when IPC was augmented with either passive twitches or active light-intensity muscular contractions, power output thereafter increased. More specifically, we observed a 3.8% increase in power with the addition of EMS to IPC and a 5% increase in power when slow walking was performed during the IPC treatment. The effect of an 11–15 W increase in max power could be quite meaningful in a competition situation, and when modeled using the current participants' weight and the assumption of zero grade and wind while cycling, these augmentations in power would be expected to result in a 0.5–0.7 kph improvement in speed (Cycling Power Lab, 2018). While it is difficult to compare directly the muscular stress while under IPC, it is likely that the added stress of walking was greater than that of EMS. It is also likely that this utilized additional muscle mass, thus, the increased efficacy with walking is logical. Furthermore, it was observed that of the 12 participants, 8 did not initially demonstrate improvements in power output with traditional IPC. However, when a greater metabolic stress was imposed, 7 of the 8 "non-responders" became "responders" and increased maximal power output, which is in line with previous evidence that a greater physiologic stimulus reduces the rate of non-response to a given perturbation (Ross et al., 2015). Comparing to previous literature, it is worth noting that the one study which previously reported no change in peak power output during cycling following IPC also used the lowest occlusion pressure (Hittinger et al., 2014), and it is thus possible that the induced metabolic stress was lower, similar to the pattern we report here.

In line with the current model of increasing the accumulation of metabolic waste products, Crisafulli et al. (2011) have similarly attempted to magnify this effect by occluding leg circulation (for 3 min) immediately following submaximal cycling exercise. In partial agreement with our findings, this group reported that IPC consistently increased peak power output compared to a control test; however, augmenting the metabolic stress through a post-exercise occlusion did not demonstrate further benefit compared to traditional IPC. This may suggest that the initial IPC provided a sufficient stimulus to elicit an optimal performance effect, or that the addition of a brief 3-min augmented IPC period was insufficient to further amplify the response. In our study, in which we invoked muscle contractions throughout all cycles of the IPC, this stress was prolonged and repeated and may account for the differences in response. While we did not observe efficacy of traditional (using similarly matched) IPC protocol and graded cycling test with male participants, the addition of metabolic stress led to a response of similar magnitude. The discrepancy regarding the efficacy of traditional IPC between studies may be attributable to IPC protocol differences or participant training status, as subjects in the current study reached ∼10% higher peak watts, and thus a higher threshold of metabolic stress during IPC may have been required to elicit a similar response. The specific role of training status on the efficacy of IPC for affecting exercise performance requires further study.

Maximal Aerobic Capacity

As is consistent with the majority of other studies, compared to the control group, there was no increase in VO_2max after traditional IPC (Bailey et al., 2012; Hittinger et al., 2014; Sabino-Carvalho et al., 2017). Despite improvements in exercise performance (peak watts), when metabolic IPC stress was augmented with the addition of either passive or active muscle contractions VO_2max remained unaltered compared to the control. This suggests that performance gains are not the result of an increase in maximal capacity. There was also no change in submaximal VO_2 during cycling following

traditional IPC, or following IPC combined with EMS or walking. This too is consistent with current literature (Clevidence et al., 2012) showing no change with traditional IPC, while also providing evidence that increasing the magnitude of the metabolic stimulus during IPC may have no effect on submaximal efficiency. Interestingly, 4 weeks of applying IPC after sprint interval training has been shown to increase VO_2max (Taylor et al., 2016), suggesting an augmented IPC stress may be important in long-term aerobic adaptation rather than a short-term change. It must be recognized that it is possible our VO_2 measures were affected by a preceding anaerobic test. If true, this is a potential explanation for the disagreement between a previous study (De Groot et al., 2010) that reported an increase in VO_2max with traditional IPC; however, this is unlikely as our findings are consistent with the majority of the existing literature (Bailey et al., 2012; Hittinger et al., 2014; Sabino-Carvalho et al., 2017).

Anaerobic Capacity

Using a standard 30 s anaerobic Wingate test, there was no change in anaerobic peak power following traditional IPC or following IPC combined with EMS or walking. This finding agrees with previous studies (Lalonde and Curnier, 2014; Paixao et al., 2014) showing no ergogenic effect of IPC on anaerobic exercise, while also providing novel evidence that the magnitude of the metabolic stimulus during IPC may have little impact on anaerobic exercise. A select few studies have shown a beneficial effect of IPC on anaerobic exercise (Patterson et al., 2015; Cruz et al., 2016), with positive effects typically occurring when IPC is employed further in advance (i.e., 30–60 min) of the exercise test; whereas studies that showed reduced or unchanged anaerobic performance used shorter periods (i.e., 5–15 min) (Lalonde and Curnier, 2014; Paixao et al., 2014) between IPC and the exercise test. Of note, the anaerobic test used in the current study occurred in the shorter time frame. The specific role of timing on the efficacy of IPC for affecting maximal anaerobic capacity needs to be further investigated. In addition, the studies that have shown positive effects of IPC on anaerobic exercise (Patterson et al., 2015; Cruz et al., 2016) appear to employ longer anaerobic effects ($\geq$60 s) compared to the studies (Lalonde and Curnier, 2014; Paixao et al., 2014) that show no effect (30 s). The current study did not show any changes in peak or average power with IPC during the first or last 10 s of the 30 s Wingate test, suggesting that IPC does not assist with short-term energy provision. It is possible that IPC assists with energy provision with longer anaerobic efforts, but this remains speculative and requires further investigation.

The specific mechanism by which IPC works remains unclear. It is possible that the combination of IPC and rhythmic muscle contractions sufficiently altered local oxygen and metabolites to activate afferent feedback leading to a metaboreflex-induced sympathetic response during exercise, while IPC alone did not. This increase in sympathetic activity to non-active muscle could lead to greater blood flow and perfusion of the active muscle beds (Boulton et al., 2018), and if preconditioning were performed locally, the proper distribution of blood flow could be further aided by sympatholysis during treatment (Horiuchi et al., 2015). Nevertheless, we observed no change in whole body VO_2max. An alternative explanation may be that IPC permits an enhanced central motor efferent command by attenuating inhibitory signals originating from metabolic sensory muscle afferents (Crisafulli et al., 2011). This would, thus, allow participants to exercise slightly beyond their individual critical threshold of exhaustion for the exercise, which fits with our finding of increased power. Indeed, a complete blockade of muscle afferent feedback during exercise, using an intrathecal administration of fentanyl, results in large increases in central motor drive and power output (Amann et al., 2011). de Oliveira Cruz et al. (2015) have observed an increase in aerobic energy provision with IPC, possibly reducing the utilization rate of anaerobic energy stores, lowering fatigue signals and delaying exhaustion. While the current study also does not offer any mechanistic insight, future studies will need to include more invasive measurements of blood flow, oxygen delivery, and arteriovenous oxygen difference across the working limb to determine whether IPC results in tissue specific improvements in these variables, which may be responsible for small improvements in peak watts.

Limitations

As with most performance research, there were potential limitations to the current study that should be recognized. The inclusion of a sham control for each IPC intervention was omitted, both for practicality and to avoid introducing a potential training effect of excessive repeated testing of the same subjects. As such, it is possible that that a placebo effect could have occurred, if participants believed the treatment would help. However, participants were naïve to the expected treatment outcomes and it is conceivable that placebo effects were no more likely to occur than nocebo effects. It is undeniable that that this area of research, as a whole has struggled to find an effective sham control, and while previous research has used low-pressure sham conditions in which the cuff is only inflated to 10–20 mmHg (Jean-St-Michel et al., 2011; Bailey et al., 2012), this low pressure is easily distinguishable from true IPC. In addition, it is still unknown if the low-pressure itself can elicit a preconditioning response, thus we chose not to employ this technique in the current study and compared to a simple control condition. Finally, the current study was conducted with participants that are young and recreationally active, thus, the relevance of these interventions in an athletic or clinical population remain to be tested.

CONCLUSION

In a group of participants for whom a traditional IPC stimulus was not effective, the amplification of an IPC stress through muscle contractions while under occlusion led to a subsequent increase in exercise performance. These findings support the

hypothesis that there needs to be a sufficient metabolic and/or hypoxic stimulus for IPC to elicit an ergogenic action. From a practical standpoint, the addition of either passive or active muscle contractions to the standard IPC protocol of 3 sets of 5-min cycles of occlusion and reperfusion, may improve the efficacy and decrease "non-response" to IPC treatment, and this highlights that new variations of the IPC protocol should be explored in an effort to optimize the desired effect. Thus, augmenting the metabolic or hypoxic stress through muscle contractions may be an important and functional way to ensure the required metabolic/hypoxic stimulus is met for IPC to improve exercise capacity.

AUTHOR CONTRIBUTIONS

JB and JS had met the guidelines for authorship, and this manuscript had been read and approved by both authors.

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Blood Flow Restriction Training Reduces Blood Pressure During Exercise Without Affecting Metaboreflex Activity

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Objective: Blood flow restriction training (BFRT) has been proposed to induce muscle hypertrophy, but its safety remains controversial as it may increase mean arterial pressure (MAP) due to muscle metaboreflex activation. However, BFR training also causes metabolite accumulation that may desensitize type III and IV nerve endings, which trigger muscle metaboreflex. Then, we hypothesized that a period of BFR training would result in blunted hemodynamic activation during muscle metaboreflex.

Methods: 17 young healthy males aged 18–25 yrs enrolled in this study. Hemodynamic responses during muscle metaboreflex were assessed by means of postexercise muscle ischemia (PEMI) at baseline (T0) and after 1 month (T1) of dynamic BFRT. BFRT consisted of 3-min rhythmic handgrip exercise applied 3 days/week (30 contractions per minute at 30% of maximum voluntary contraction) in the dominant arm. On the first week, the occlusion was set at 75% of resting systolic blood pressure (always obtained after 3 min of resting) and increased 25% every week, until reaching 150% of resting systolic pressure at week four. Hemodynamic measurements were assessed by means of impedance cardiography.

Results: BFRT reduced MAP during handgrip exercise (T1: 96.3 ± 8.3 mmHg vs. T0: 102.0 ± 9.53 mmHg, $p = 0.012$). However, no significant time effect was detected for MAP during the metaboreflex activation ($P > 0.05$). Additionally, none of the observed hemodynamic outcomes, including systemic vascular resistance (SVR), showed significant difference between T0 and T1 during the metaboreflex activation ($P > 0.05$).

Conclusion: BFRT reduced blood pressure during handgrip exercise, thereby suggesting a potential hypotensive effect of this modality of training. However, MAP reduction during handgrip seemed not to be provoked by lowered metaboreflex activity.

Keywords: blood flow restriction, ischemia, metaboreflex, exercise training, exercise pressor reflex, blood pressure

INTRODUCTION

Resistance training with low to moderate loads performed under blood flow restriction (BFR) has been shown to elicit muscle hypertrophy and strength gains (Kaijser et al., 1990; Abe et al., 2006; Manini and Clark, 2009; Takada et al., 2012). These effects have been associated to increased metabolite accumulation in the active skeletal muscle (Suga et al., 2012; Pearson and Hussain, 2015). On the other hand, the metabolite accumulation due to BFR also increases afferent signaling of group IV afferent muscle nerves (Nobrega et al., 2014). Together with central command and arterial/cardiopulmonary baroreceptors, groups III and IV skeletal muscle nerve afferents play an important role in mediating hemodynamic responses to exercise.

In addition, cardiovascular reflexes from group IV skeletal muscle afferents appear to be dysregulated in several cardio-metabolic diseases, such as obesity, metabolic syndrome, type 2 diabetes mellitus, chronic heart failure, or hypertension (Crisafulli et al., 2007, 2013; Piepoli et al., 2008; Sausen et al., 2009; Delaney et al., 2010; Roberto et al., 2012). The over-activation of signals originating from muscle type IV nerve endings has been suggested to be one of the beneficial effects of regular exercise upon chronic heart failure (Crisafulli et al., 2007, 2013; Piepoli et al., 2008). In fact, exercise training has been reported to reduce the metaboreflex activity (Wang et al., 2010).

Different approaches have been used to assess muscle metaboreflex activity (Alam and Smirk, 1937, 1938; Rowell et al., 1986; Crisafulli, 2017). In general, they involve BFR to the exercising muscles during or postexercise, thereby causing a mismatch in the oxygen supply-to-demand ratio. Consequently, an increased accumulation of metabolites occurs with the activation of group IV muscle afferents. Under physiological conditions, postexercise muscular ischemia (PEMI) activates afferent signals of group IV afferents in isolation from central command and muscle mechanoreflex (Crisafulli et al., 2011).

We could not find prior studies investigating the effects of BFR training on the hemodynamic responses induced by the metaboreflex. However, a recent study suggested that the exercise pressor reflex would be lowered after BFR training, therefore reducing the heart rate (HR) and blood pressure during the metaboreflex (Sundblad et al., 2018). Additionally, some other trials reported that BFR might have an acute hypotensive effect (Neto et al., 2015). However, potential mechanisms of hypotension after BFR training have not been investigated. In short, there is a lack of research explaining the cardiovascular effects of BFR training.

Considering the exercise-related reduction in the metaboreflex activity, as well as relatively recent findings in regard to blood pressure reduction after BFR, it is possible to speculate that a period of BFR training would result in attenuated blood pressure during the muscle metaboreflex activation. These data would have the following practical applications: (a) to help detecting hemodynamic improvements due to BFR training; (b) to demonstrate the safety of chronic BFR training and describe its hemodynamic effects.

Thus, the present study investigated whether BFR training would be capable to reduce the blood pressure and improve the vascular response during muscle metaboreflex activation in healthy subjects. We tested the hypothesis that a possible blood pressure reduction within metaboreflex activation would be due to lowered systemic vascular resistance (SVR). Additionally, we tested the hypothesis that BFR training might reduce the vasoconstriction mediated by the metaboreflex.

MATERIALS AND METHODS

Sample

Sample size calculation was performed using the G-power software (Faul et al., 2007). A sample of 16 individuals was determined for an error probability of 0.05, effect size of 0.8, and power of 0.90 ($1 - \beta$). Initially, 39 volunteers enrolled in the study. Of these, 15 were excluded for not completing at least 75% of the planned training sessions. Another seven subjects were excluded due to low quality in their cardio-impedance tracing signals. Therefore, data of 17 male volunteers aged 18–25 years (21 ± 2 years; 1.73 ± 0.06 m; 76.3 ± 11.6 kg) were retained in the final analysis. All subjects were normotensive [117 ± 10 mmHg and 82 ± 9 mmHg (resting mean $\pm$ SD systolic and diastolic blood pressures, respectively)] and regularly practicing physical activity. None of the volunteers had history of cardiorespiratory or metabolic diseases or were under medications or supplements that might affect autonomic and hemodynamics responses. All participants signed informed consents and the experiment gained approval from the ethics board committee of the University of Rio de Janeiro State (process 69072916.8.0000.5282).

Experimental Design

All subjects had hemodynamic responses assessed at rest, during handgrip exercise and during PEMI (metaboreflex activation), before (T0) and after 4 weeks (T1) of low intensity BFR training. All assessments took place within 1–3 days before T0 and after T1.

After a general medical examination, subjects remained 10 min at rest in a sit position before PEMI and control exercise recovery (CER) protocols. PEMI and CER were performed in a counterbalanced random order, with 10 min of resting between them. In the PEMI protocol, after 3 min of resting, the individuals performed rhythmic dynamic handgrip for another 3 min (30 contractions per min) with load corresponding to 30% of maximum voluntary contraction. Subsequently, 3 min of PEMI on the exercised arm was applied, by means of rapidly inflation (<3 s) of a tourniquet placed at the upper arm up to 50 mmHg above peak exercise systolic pressure. The cuff was inflated just at the cessation of exercise and was kept inflated for 3 min. After deflating the cuff, the individuals underwent additional 3 min recovery (total of 6 min). A manual sphygmomanometer (Welch^{TM} AllymTM DS44, Skaneateles Falls, NY, United States) was used to asses systolic and diastolic blood pressures (SBP and DPB) at every minute during, always in the non-dominant arm. This protocol has been shown to trap muscle metabolites in the exercising limb and to maintain stimulation of the metaboreceptors (Crisafulli et al., 2003, 2006, 2007, 2008). Moreover, this procedure allows isolating the metaboreflex activity from the activity due to central command

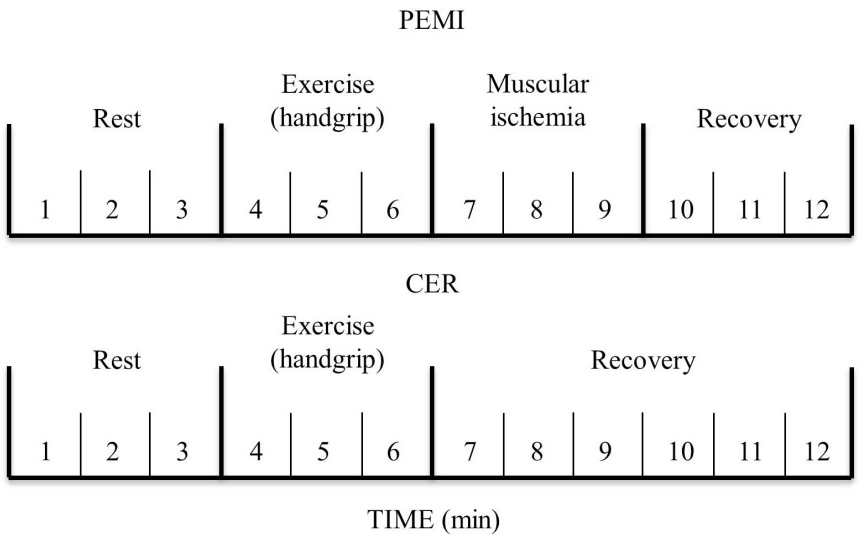

FIGURE 1 | Metaboreflex assessment.

and mechanoreflex activation, since during PEMI these two cardiovascular reflexes are not operating (Bastos et al., 2000; Crisafulli et al., 2011). In the CER protocol, the same rest-exercise protocol used for PEMI was performed, followed by 6-min recovery without tourniquet inflation (**Figure 1** – Metaboreflex assessment). All experiments took place in the morning, in a temperature- controlled room (22°C, relative humidity 50%).

Blood Flow Restriction (BFR) Training

Prior to the experimental conditions, maximal handgrip values were individually obtained through 5 maximum attempts with duration of 5 s interspersed with 1 min intervals. All measurements were recorded by using a previously calibrated MP150 Data Acquisition Systems module and the AcqKnowledge software (BIOPAC^{TM} Systems, Inc., Goleta, CA, United States). After all attempts, the maximal value was used to calculate the handgrip training load. One to 3 days after PEMI and CER assessments at baseline, the individuals began the supervised BFR training, 3 days per week during 4 weeks (therefore completing 12 sessions). As aforementioned, after 3 min seated at rest, 3 min of rhythmic handgrip exercise (30 contractions per min at 30% of maximum voluntary contraction) were performed with BFR being applied at the exercised arm. A visual inspection was allowed to the subjects in order to control their handgrip strength by means of the AcqKnowledge software. On the first week, the occlusion was set at 75% of resting SBP (always obtained after 3 min of resting) and increased 25% every week, until reaching 150% of resting SBP at week four.

Hemodynamic Assessment

Hemodynamic assessments were performed by means of impedance cardiography (New Core^{TM}, 2C Technologies Inc., Cagliari, Italy). The impedance method has been previously used in similar experimental settings (Crisafulli et al., 2003, 2007, 2011), and data acquisition procedures were described in detail in previous works by our group (Crisafulli et al., 2003, 2008). In short, the New Core device recorded impedance (Z_0) and ECG traces on a secure digital memory card. The recorded Z_0 and ECG traces were analyzed offline employing a digital chart recorder (ADInstruments^{TM}, PowerLab 8sp, Castle Hill, Australia). The Z_0 first derivative (dZ/dt) was calculated and the Sramek–Bernstein equation (Crisafulli et al., 2006) was employed to calculate beat-to-beat stroke volume (SV) values, as follows: $SV = (VEPT \bullet Z_0^{-1}) \bullet dZ/dt_{max} \bullet VET$; where VEPT is the volume of electrical participating tissue (determined using a nomogram based on sex, height, and body mass); Z_0 is the thorax impedance at the end of cardiac diastole; dZ/dt_{max} is the maximal Z_0 first derivative during cardiac systole; and VET is the left ventricular ejection time, calculated as the interval between the beginning and minimum deflection of dZ/dt trace during systole.

The pre-ejection period (PEP) was assessed as the time interval between the onset of electrocardiogram Q wave and the beginning of the widest deflection occurring in the dZ/dt trace (Crisafulli et al., 2000, 2003). The HR was calculated as the reciprocal of the electrocardiogram R–R interval and the cardiac output (CO) was obtained by multiplying SV and HR. Furthermore, diastolic time (DT) was measured by subtracting the sum of PEP and VET from the total cardiac cycle period. The mean systolic ejection rate (VER), which is an index of myocardial performance, was obtained by calculating the SV/VET ratio (Gledhill et al., 1994; Sanna et al., 2017). The ventricular filling rate (VFR), which is a measure of the mean rate of diastolic blood flux, was calculated by dividing SV by DT (Sanna et al., 2017).

The individuals were also connected to a standard manual sphygmomanometer (Welch Allym^{TM} DS44, Skaneateles Falls, NY, United States) to assess SBP and DBP, always in the non-dominant arm by the same trained researcher. The mean arterial blood pressure (MAP) was calculated using the formula proposed by Moran and co-workers (Moran et al., 1995). The systemic

vascular resistance (SVR) was obtained by multiplying the MAP/CO ratio by 80, where 80 is a conversion factor to change units to standard resistance units. Blood pressure measurements were taken every minute by a single and experienced researcher.

Data Analysis

The Shapiro-Wilk test revealed that all measured variables were normally distributed. Data are presented as mean ± SD. Hemodynamic data during PEMI and CER tests were averaged over 1 min. Values at the third minute of rest, at the third minute of exercise, and at the third minute of recovery in both protocols were considered for statistical analysis. In order to assess the metaboreflex activity, differences of all variables between PEMI and CER at the third minute of recovery were calculated (Crisafulli et al., 2013). Differences of outcomes between experimental situations were tested by 2-way ANOVA with repeated measures (factors: time and condition) followed by Bonferroni *post hoc* tests in the event of significant F ratios, while differences in deltas (Δ) for each main variable responses between T0 and T1 were assessed by t-tests for paired data. All calculations were performed using a commercially available software (GraphPad™, Prism, La Jolla, CA, United States), and in all cases statistical significance was set at $P \leq 0.05$.

RESULTS

Table 1 depicts resting hemodynamic outcomes before PEMI and CER, at T0 and T1. There was no significant difference across conditions and between T0 and T1 for HR, CO, MAP, SVR, VER, or VFR. A significant time effect (T0 vs. T1) was observed only for SV, which lowered after BFR training.

Table 2 presents data of main hemodynamic variables at the third minute of exercise in PEMI and CER, also at T0 and T1. Significant differences were not detected for HR, CO, SV, SVR, VFR, and VER between experimental conditions and between T0 and T1. On the other hand, a significant reduction in MAP was observed at T1 *vs.* T0 in both PEMI (101.7 ± 11.4 vs. 96.3 ± 8.3 mmHg) and CER (102.0 ± 9.5 vs. 95.7 ± 8.1 mmHg, $P = 0.012$ for time factor).

Figures 2, **3** exhibit hemodynamic outcomes obtained during the third minute of recovery at T0 and T1 for PEMI and CER, as well as their responses due to metaboreflex activity. **Figure 2A** shows that there was no difference due to condition (PEMI and CER) or time (T0 and T1) for HR, nor HR response was different between T0 and T1 (**Figure 2B**). Similarly, SV was not affected by condition or time (**Figure 2C**) and its response was similar at T0 and T1 (**Figure 2D**). Consequently, CO remained stable by condition and time (**Figure 2E**), with similar responses being observed at T1 and T0 (**Figure 2F**). **Figure 3A** shows that MAP was significantly lower during CER vs. PEMI ($P = 0.044$ for condition), without any significant time effect. Moreover, MAP (**Figure 3B**) was not different between T0 and T1. SVR (**Figure 3C**) was not influenced by time or condition, nor its response was different between T0 and T1 (**Figure 3D**). Similarly, VER and VFR were not affected by time and condition (**Figures 3E,G**) and their responses to metaboreflex were similar at T0 and T1 (**Figures 3F,H**).

DISCUSSION

This study investigated whether 1 month of BFR training was capable to reduce blood pressure during muscle metaboreflex activation in healthy subjects. Our findings indicate that training with BFR reduced MAP during dynamic handgrip (please refer to **Table 2**). This finding was in line with recent observations reporting hypotensive effects of BFR (Araujo et al., 2014; Maior et al., 2015; Neto et al., 2015, 2017; Sundblad et al., 2018). However, our results did not confirm the premise that MAP

TABLE 1 | Resting hemodynamic data before (T0) and after (T1) blood flow restriction in PEMI and CER (mean ± SD) (n = 17).

	T0	T1	*p*-value time effect	*p*-value condition effect	*p*-value interaction
HR (bpm)	PEMI 66.6 ± 9.2 CER 68.1 ± 11.5	PEMI 71.5 ± 11.7 CER 70.3 ± 10.8	0.181	0.954	0.609
SV (ml)	PEMI 135.5 ± 26.1 CER 122.3 ± 32.8	PEMI 115.5 ± 27.3* CER 112.8 ± 22.3*	0.028	0.230	0.441
CO ($L \cdot min^{-1}$)	PEMI 9.01 ± 2.05 CER 8.36 ± 2.76	PEMI 8.28 ± 2.17 CER 7.87 ± 1.62	0.254	0.321	0.821
MAP (mmHg)	PEMI 96.4 ± 10.9 CER 95.0 ± 9.7	PEMI 91.5 ± 7.4 CER 92.8 ± 9.7	0.128	0.982	0.560
SVR (dynes $\cdot s^{-1} \cdot cm^{-5}$)	PEMI 892.3 ± 202.7 CER 1004.8 ± 348.1	PEMI 957.9 ± 282.7 CER 977.8 ± 198.4	0.765	0.307	0.474
VER ($ml \cdot s^{-1}$)	PEMI 399.8 ± 73.1 CER 397.9 ± 89.3	PEMI 374.4 ± 85.0 CER 376.3 ± 86.1	0.253	0.990	0.931
VFR ($ml \cdot s^{-1}$)	PEMI 374.0 ± 249.0 CER 330.7 ± 183.2	PEMI 330.4 ± 188.3 CER 331.1 ± 188.9	0.664	0.668	0.658

*HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; SVR, systemic vascular resistance; VER, mean systolic ejection rate; VFR, ventricular filling rate. *Significantly different vs. T0.*

TABLE 2 | Hemodynamic data values during the third minute of exercise (dynamic handgrip) of PEMI and CER tests before (T0) and after blood flow restriction protocol (T1).

	T0	T1	*p*-value time effect	*p*-value condition effect	*p*-value interaction
HR (bpm)	PEMI 69.6 ± 10.5 CER 71.3 ± 12.5	PEMI 73.4 ± 12.0 CER 75.0 ± 12.8	0.201	0.572	0.986
SV (ml)	PEMI 118.9 ± 27.9 CER 117.7 ± 32.5	PEMI 116.8 ± 29.3 CER 112.8 ± 24.9	0.617	0.710	0.841
CO ($L \cdot min^{-1}$)	PEMI 8.23 ± 2.00 CER 8.45. ± 2.86	PEMI 8.41 ± 1.88 CER 8.33 ± 1.73	0.954	0.894	0.775
MAP (mmHg)	PEMI 102.0 ± 9.53 CER 101.7 ± 11.4	PEMI 96.3 ± 8.3* CER 95.7 ± 8.1*	0.012	0.947	0.844
SVR ($dynes \cdot s^{-1} \cdot cm^{-5}$)	PEMI 1073.8 ± 378.2 CER 1041.4 ± 279.9	PEMI 962.6 ± 238.6 CER 953.3 ± 210.1	0.152	0.867	0.763
VER ($ml \cdot s^{-1}$)	PEMI 369.1 ± 89.3 CER 387.2 ± 98.8	PEMI 381.1 ± 93.1 CER 378.9 ± 86.8	0.934	0.723	0.651
VFR ($ml \cdot s^{-1}$)	PEMI 351.3 ± 170.2 CER 341.4 ± 193.9	PEMI 341.5 ± 121.2 CER 365.2 ± 193.6	0.867	0.869	0.689

*Values are mean ± SD. N = 17. HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; SVR, systemic vascular resistance; VER, mean systolic ejection rate; VFR, ventricular filling rate. *Significantly different vs. T0.*

reduction during exercise would be due to lowered metaboreflex activity. Actually, no significant time effect was found for MAP during metaboreflex activation (**Figure 3A**).

Moreover, the MAP response calculated as the difference in MAP between PEMI and CER was not different between T0 and T1 (please refer to **Figure 2B**). Although a tendency for MAP reduction occurred when comparing T1 vs. T0 (+2.2 ± 4.6 vs. +4.0 ± 3.8), this difference was not statistically significant ($P = 0.282$). No other cardiovascular outcome was influenced by the metaboreflex activation, before or after BFR training (T1 vs. T0). These data reject the hypothesis of a blunted metaboreflex activity due to BFR training, and do not support any effect of BFR training upon muscle metaboreflex activity, at least in young and healthy male subjects.

The second objective of this experiment was to verify whether BFR training would reduce SVR and cause a shift from vasoconstriction- to flow-mediated mechanism through which the target blood pressure would be achieved during the metaboreflex activation. This initial hypothesis was also rejected, since SVR remained unaltered after training. However, it is possible to speculate that the lowered MAP during handgrip was probably due to a slight reduction in SVR at T1 vs. T0, although statistical significance was not reached (**Table 2**, $P = 0.152$). Further research with larger sample sizes to prevent type II error is warranted to clarify this point.

We do not have any definitive explanation for the reduced MAP during handgrip at T1 vs. T0. As previously said, this phenomenon seemed not to be mediated by the metaboreflex. It is though possible to think that the set point of baroreflex activity for the blood pressure regulation during exercise has been somehow modified by the BFR training. It has been reported that exercise training may decrease the sympathetic activity, by improving the baroreceptor control in healthy individuals, as well as in patients with cardiovascular disease (Krieger et al., 2001; La Rovere and Pinna, 2014). In the present study, it is therefore feasible to hypothesize that after BFR training the baroreflex has been more effective in buffering the exercise-related increase in sympathetic activity (Sausen et al., 2009; Delaney et al., 2010). However, we could not find previous studies investigating the effects of BFR training upon baroreflex activity. Additional research is needed to confirm this possibility.

This is probably the first study demonstrating a potential hypotensive effect of BFR training. Some previous trials have reported acute hypotension after different protocols using BFR (Araujo et al., 2014; Maior et al., 2015; Neto et al., 2015), but none addressed the chronic hypotensive effect of this modality of training. Our findings suggested that BFR training might be effective in lowering blood pressure during exercise. These data have potential clinical application in the treatment of hypertension, which should be further investigated in patients with high blood pressure.

An unexpected result was the significant reduction of resting SV at T1 in comparison with T0. We cannot provide any explanation for this outcome, since we could not find prior studies that investigated the chronic effects of BFR training upon this specific variable. However, reductions in SV have been reported during acute ischemic training (Iida et al., 2007; Downs et al., 2014; Sprick and Rickards, 2017), and some authors speculated that this effect might result from a venous pooling inhibiting venous return (Iida et al., 2007). Whatever the cause, the decrease in SV at rest seemed to be compensated by a slight increase in HR, so that CO remained unchanged.

The safety of BFR training has been questioned (Wernbom et al., 2008; Spranger et al., 2015) due to potential muscle damage, thrombosis, endothelial dysfunction, or excessive increase in blood pressure. Our results did not support the presupposition that ischemic training would be dangerous, at least when following the characteristics of the present protocol. Actually, any of these problems have been detected or reported during the intervention.

The major limitation of the present study was the relatively small sample size ($n = 17$) that completed the

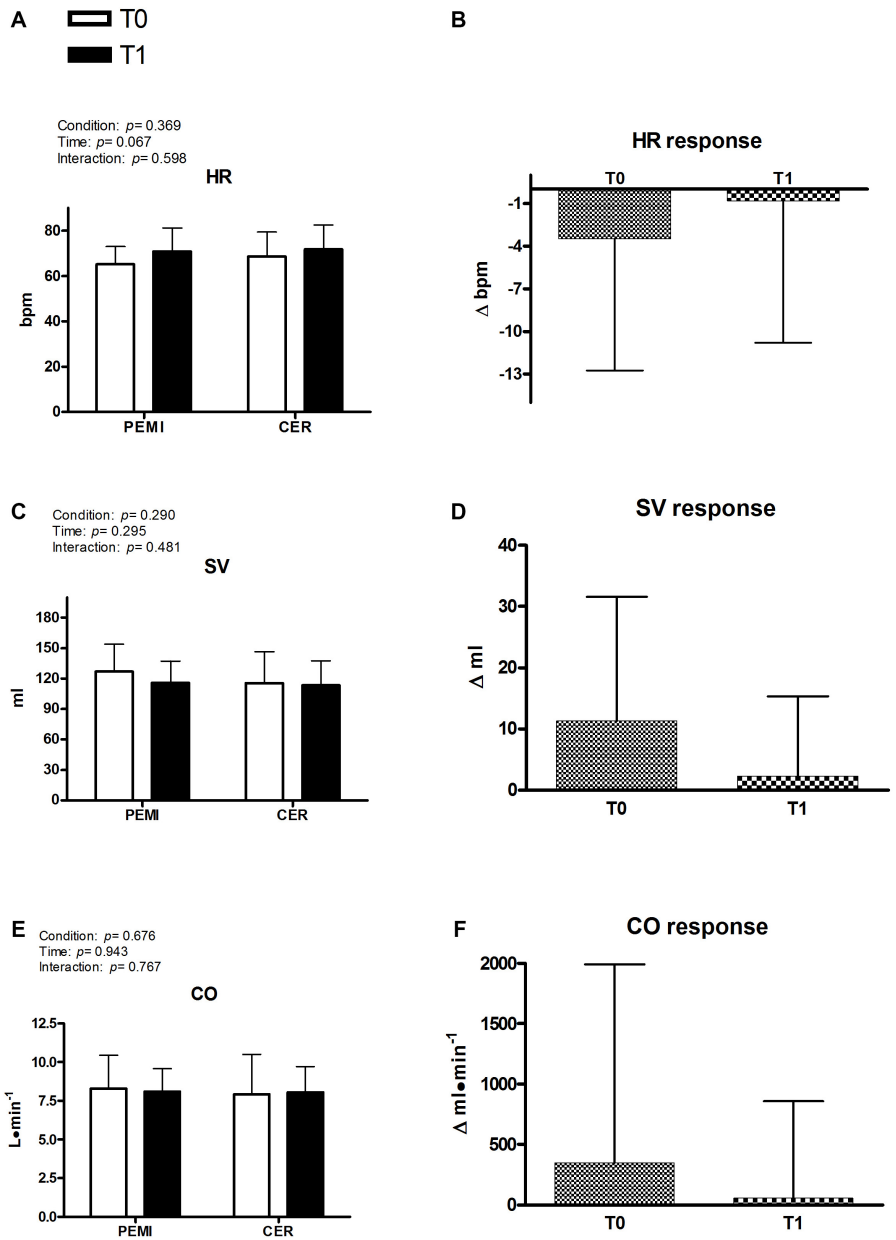

FIGURE 2 | Absolute values during post-exercise muscle ischemia (PEMI) and control exercise recovery (CER) tests obtained during the third minute of recovery and response in heart rate (HR, **A,B**), stroke volume (SV, **C,D**), and cardiac output (CO, **E,F**) before (T0) and after (T1) a period of training with blood flow restriction (n = 17).

BRF training protocol, which prevented a better evaluation of the hemodynamic consequences of ischemic training and, in some cases, may have introduced type II error (as in the case of SVR reduction). Moreover, only men were included in our sample. We made this choice to avoid bias related to hormonal changes during the menstrual cycle, which could affect vascular responsiveness and interfere with the metaboreflex response. On the other hand, this feature limits the potential generalization of our data. The lack of a formal control group could be also considered as a methodological limitation. However, given that our main goal was to test the acute hemodynamic responses following PEMI and CER protocols, we considered that the biological test-retest evaluation would be enough – in other words, we adopted a within-individual design using the CER protocol as a control for PEMI. A formal control group composed by different subjects would add little information to the present data and might introduce inter-individual biases. Finally, it should be recognized that the fact that the individuals in the sample were physically

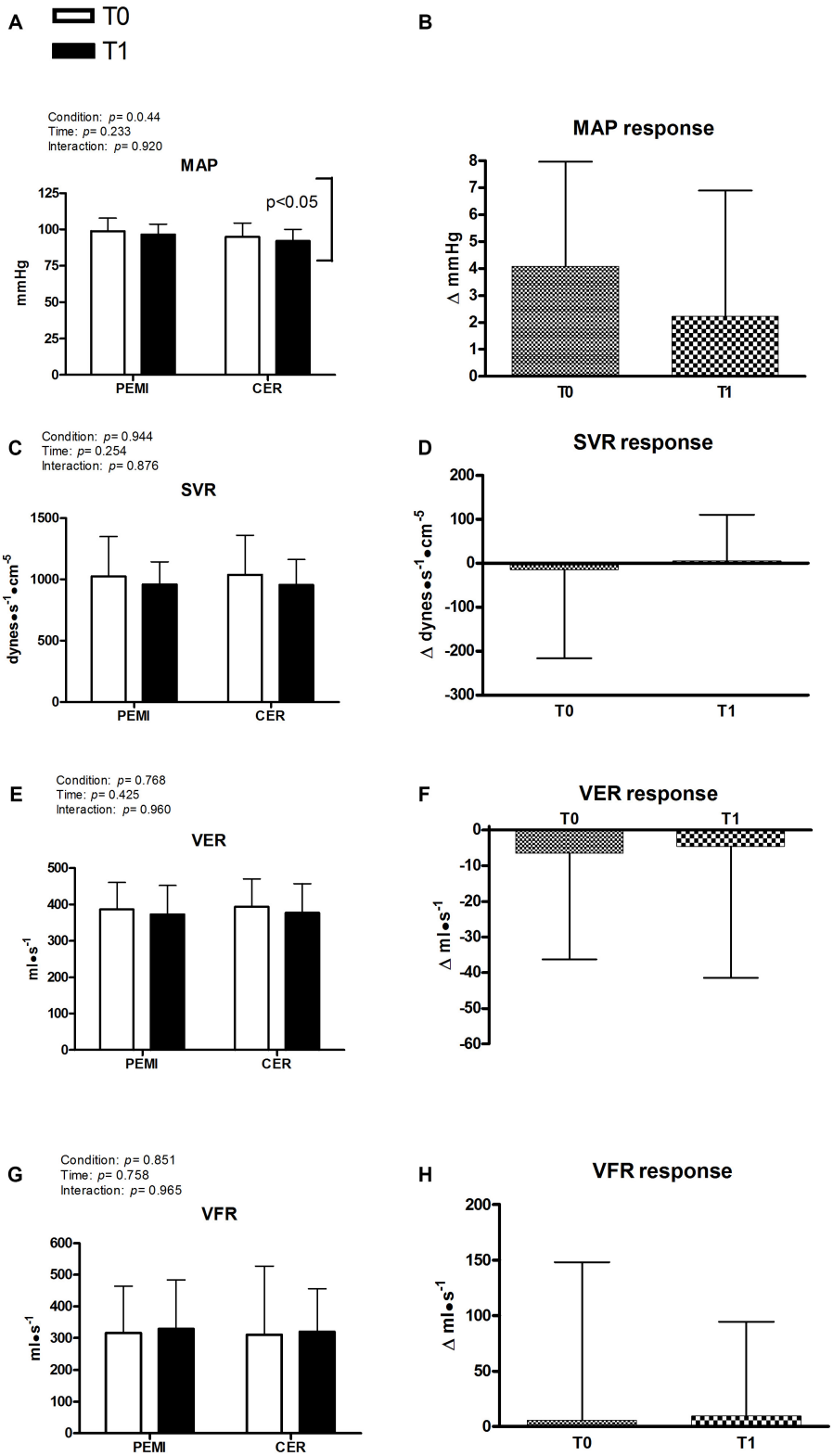

FIGURE 3 | Absolute values during post-exercise muscle ischemia (PEMI) and control exercise recovery (CER) tests obtained during the third minute of recovery and response in mean arterial pressure (MAP, **A,B**), systemic vascular resistance (SVR, **C,D**), ventricular emptying rate (VER, **E,F**), and ventricular filling rate (VFR, panels **G,H**) before (T0) and after (T1) a period of training with blood flow restriction. Values are mean ± SD (n = 17). A vertical bracket indicates the overall main effect of condition (PEMI vs. CER). There was no interaction effect.

active may have had some influence on our results. One could argue that their current exercise habits could have possibly masked the effects of BRF training upon the observed outcomes. However, considering the specificity of physiological and metabolic demands induced by successive and prolonged static contractions under BRF, it is possible to think that the general routine of physical activities of the participants was not able to modify the hemodynamic responses mediated by the metaboreflex.

CONCLUSION

In conclusion, 1 month of ischemic training did not change the metaboreflex activity. MAP and SVR at rest remained unchanged after BFR training. On the other hand, the blood pressure during handgrip reduced after ischemic training, thereby suggesting a potential hypotensive effect of this training modality. Further research with larger sample sizes is warranted to better clarify the hemodynamic consequences of ischemic training and its potential application in patients with high blood pressure.

AUTHOR CONTRIBUTIONS

All authors contributed to data analysis and interpretation, as well as to drafting and revising the manuscript. The original study design was made by AC, PF, and RO and discussed with the other authors. AC, RO, and SR performed the data analysis.

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Skeletal Muscle Mitochondrial Protein Synthesis and Respiration Increase With Low-Load Blood Flow Restricted as Well as High-Load Resistance Training

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Purpose: It is well established that high-load resistance exercise (HLRE) can stimulate myofibrillar accretion. Additionally, recent studies suggest that HLRE can also stimulate mitochondrial biogenesis and respiratory function. However, in several clinical situations, the use of resistance exercise with high loading may not constitute a viable approach. Low-load blood flow restricted resistance exercise (BFRRE) has emerged as a time-effective low-load alternative to stimulate myofibrillar accretion. It is unknown if BFRRE can also stimulate mitochondrial biogenesis and respiratory function. If so, BFRRE could provide a feasible strategy to stimulate muscle metabolic health.

Methods: To study this, 34 healthy previously untrained individuals (24 ± 3 years) participated in BFRRE, HLRE, or non-exercise control intervention (CON) 3 times per week for 6 weeks. Skeletal muscle biopsies were collected; (1) before and after the 6-week intervention period to assess mitochondrial biogenesis and respiratory function and; (2) during recovery from single-bout exercise to assess myocellular signaling events involved in transcriptional regulation of mitochondrial biogenesis. During the 6-week intervention period, deuterium oxide (D_2O) was continuously administered to the participants to label newly synthesized skeletal muscle mitochondrial proteins. Mitochondrial respiratory function was assessed in permeabilized muscle fibers with high-resolution respirometry. Mitochondrial content was assessed with a citrate synthase activity assay. Myocellular signaling was assessed with immunoblotting.

Results: Mitochondrial protein synthesis rate was higher with BFRRE (1.19%/day) and HLRE (1.15%/day) compared to CON (0.92%/day) ($P < 0.05$) but similar between exercise groups. Mitochondrial respiratory function increased to similar degree with both exercise regimens and did not change with CON. For instance, coupled respiration supported by convergent electron flow from complex I and II increased 38% with BFRRE and 24% with HLRE ($P < 0.01$). Training did not alter citrate synthase activity compared to CON. BFRRE and HLRE elicited similar myocellular signaling responses.

Conclusion: These results support recent findings that resistance exercise can stimulate mitochondrial biogenesis and respiratory function to support healthy skeletal muscle and whole-body metabolism. Intriquingly, BFRRE produces similar mitochondrial adaptations at a markedly lower load, which entail great clinical perspective for populations in whom exercise with high loading is untenable.

Keywords: ischemic resistance training, deuterium oxide, bioenergetics, high-resolution respirometry, mitochondrial biogenesis

INTRODUCTION

Aging, prolonged inactivity, and several chronic diseases negatively affect skeletal muscle mitochondrial and myofibrillar properties (Clark, 2009; Zizola and Schulze, 2013; Gram et al., 2014; Rontoyanni et al., 2017; Granata et al., 2018; Holloway et al., 2018). These negative effects may impair mobility and lead to the development of metabolic disorders such as diabetes and insulin insensitivity (Kelley et al., 2002; Schrauwen-Hinderling et al., 2007; Hesselink et al., 2016). Accordingly, strategies to improve mitochondrial and myofibrillar properties are important for skeletal muscle function and whole-body health.

Endurance-type exercise is traditionally used to stimulate mitochondrial biogenesis and to improve mitochondrial function while resistance-type exercise is traditionally used to stimulate myofibrillar accretion and strength gains (Garber et al., 2011). However, concurrent practice by traditionally recommended training principles to achieve both types of adaptations entails high exercise intensity and investment of substantial exercise time, which may be untenable in clinical populations. Interestingly, emerging evidence suggests that traditional high-load resistance exercise (HLRE) can also stimulate mitochondrial protein fractional synthesis rate (FSR) (Wilkinson et al., 2008; Donges et al., 2012). Furthermore, HLRE has been shown to improve mitochondrial respiratory function in permeabilized muscle fibers (Pesta et al., 2011; Salvadego et al., 2013; Porter et al., 2015; Holloway et al., 2018). However, it should be noted that two studies have failed to demonstrate a similar positive effect in isolated mitochondria (Irving et al., 2015; Robinson et al., 2017). This discrepancy may be attributed to disruption of mitochondrial morphology inherent to studies on isolated mitochondria (Picard et al., 2011a), which warrant further investigation into the effect of resistance exercise on mitochondrial respiratory function in permeablized fibers as well as isolated mitochondria. Collectively, these recent observations suggest that HLRE can in fact produce adaptations of importance to both myofibrillar and mitochondrial properties. Still, several clinical conditions, such as arthritis, post-surgical recovery, and advanced aging may prohibit the use of resistance exercise with high loading. Under such circumstances, blood flow restricted resistance exercise (BFRRE) could constitute a time-efficient low-load alternative. In accordance, when combined with partial restriction of blood flow, loading as low as 20–30% of maximal loading has been observed highly efficient to produce muscle hypertrophy with little time expenditure required (Fahs et al., 2015; Farup et al., 2015). However, while BFRRE is effective in stimulating muscle accretion, it is currently unknown if this approach, similar to HLRE, can also stimulate adaptations of importance to mitochondrial properties (Groennebaek and Vissing, 2017). Yet, since external restriction of blood flow during resistance exercise has been shown to augment tissue deoxygenation (Ganesan et al., 2015; Lauver et al., 2017), there is reason to believe that BFRRE could effectively stimulate such mitochondrial adaptations. In accordance ischemia is associated with perturbations in ATP turnover and reactive oxygen species (ROS) production (Korthuis et al., 1985; Kudo et al., 1995), which in turn can promote signaling for transcription of mitochondrial genes (Irrcher et al., 2009; Hood et al., 2016).

The purpose of the current study was to investigate skeletal muscle mitochondrial adaptations to 6 weeks of BFRRE and HLRE, practiced in accordance with commonly recommended guidelines (American College of Sports Medicine [ACSM], 2009; Scott et al., 2015), in young healthy subjects. Mitochondrial adaptations were assessed by using deuterium oxide (D_2O) to assess long-term mitochondrial protein FSR as well as by measuring changes in mitochondrial content, and mitochondrial respiratory function. In addition, we measured myocellular signaling after a single bout of exercise to obtain information on the stresses driving mitochondrial adaptations to BFRRE and HLRE. We hypothesized that both BFRRE and HLRE would stimulate mitochondrial adaptations and that BFRRE would further augment such adaptations compared to HLRE.

Abbreviations: 4o, state 4 respiration with oligomycin; ACC, acetyl-CoA carboxylase; AMP, adenosine monophosphate; AMPK, 5′ AMP-activated protein kinase; ANOVA, analysis of variance; AOP, arterial occlusion pressure; ATP, adenosine triphosphate; BFRRE, blood flow restricted resistance exercise; CaMKII, calcium/calmodulin dependent protein kinase II; CI, confidence interval; CON, non-exercise control; CREB, cAMP response-element binding protein; D_2O, deuterium oxide; E, maximal uncoupled respiration with FCCP; FCCP, carbonylcyanide-4-trifluoromethoxyphenylhydrazone; FSR, fractional synthesis rate; GM, leak respiration with glutamate and malate; GM3, state 3 respiration with glutamate, malate; GMS3, state 3 respiration with glutamate, malate and succinate; HLRE, high-load resistance exercise; MIDA, mass isotopomer distribution analysis; p38 MAPK, p38 mitogen-activated protein kinase; PVDF, polyvinylidene difluoride; QQ-plot, quantile-quantile plot; RCR, respiratory control ratio; RM, repetition maximum; ROS, reactive oxygen species; SD, standard deviation; SDS-PAGE, sodium dodecyl sulfate poly-acrylamide gel electrophoresis.

MATERIALS AND METHODS

Subjects

Thirtyfour young, healthy, untrained men volunteered to participate in the study. Participants were excluded from the study if they had engaged in resistance training 6 months

TABLE 1 | Baseline characteristics.

	CON	BFRRE	HLRE
n	10	12	12
Age (yr)	24 (21; 27)	23 (22; 24)	24 (23; 26)
Height (cm)	182.7 (176.9; 188.4)	180.5 (177.2; 183.7)	179.1 (176.2; 181.9)
Weight (kg)	79.1 (71.7; 86.5)	72.2 (66.3; 78.1)	82.0 (71.3; 92.7)
BMI (kg/m^2)	23.8 (21.4; 26.1)	22.2 (20.5; 23.8)	25.54 (22.4; 28.7)

BMI, body mass index; BFRRE, blood flow restricted resistance exercise; CON, non-exercise control; HLRE, high-load resistance exercise. Data are shown as means with 95% CI.

prior to inclusion and/or if they had participated in other structured moderate/high intensity exercise training (>1 h week^{-1}) 6 months prior to inclusion. Other exclusion criteria included routine strenuous work- and/or commute-related physical activity, use of prescriptive medicine with known or potential effects on muscle metabolism and growth, and intake of dietary supplements (e.g., protein and creatine supplements). Baseline subject characteristics are presented in **Table 1**.

Written informed consent was obtained from all participants prior to inclusion. The study was approved by the Central Denmark Region Committee on Health Research Ethics (1-10-72-218-16) and registered in the database clinicaltrials.gov (NCT03380663). The study conformed to the standards for human experimental trials outlined in the Declaration of Helsinki.

Study Design

Participants were randomly assigned to BFRRE (n = 12), HLRE (n = 12), or a non-exercise control group (CON) (n = 10). For each participant, the total study length was 9 weeks and comprised participation in a single-trial study and a long-term study (**Figure 1**). All participants completed both the single-trial study and the long-term study.

The single-trial study was conducted to evaluate acute myocellular signaling involved in transcriptional regulation of mitochondrial adaptations. Muscle biopsies were harvested immediately (at 0 h) and 3 h after a single bout of the prescribed intervention. The long-term study was conducted to evaluate cumulative mitochondrial adaptations to 6 weeks of BFRRE and HLRE. For BFRRE and HLRE, the 6-week intervention period comprised a total of 18 exercise sessions, which has previously been demonstrated to be sufficient to promote increases in muscle functional capacity and muscle growth (Farup et al., 2015; Jakobsgaard et al., 2018). Training compliance was 100% in the BFRRE group and 98.1% in the HLRE group. 5 days before the single trial study and 4 days after cessation of the 6-week intervention period, subjects reported to the laboratory for muscle biopsies and exercise capacity testing. The CON group performed all experimental procedures except exercise.

Throughout the study period, subjects were instructed not to deviate from their habitual physical activity level. Tests of basal exercise capacity and biopsy samplings were conducted at the same absolute time points in the early morning after overnight fasting to preclude potential influence of circadian rhythm and food intake. The subjects were instructed to refrain from strenuous physical activity and alcohol 3 days prior to all tests.

Deuterium Oxide Administration

D_2O (99.8%, Sigma Aldrich, St. Louis, MO, United States) was orally administered to measure long-term mitochondrial protein FSR using a modified approach from our previous studies (**Figure 1**; Scalzo et al., 2014; Miller et al., 2015; Konopka et al., 2017). Specifically, during an initial D_2O loading period (week 3), subjects received 2 × 1 mL per kg bodyweight during day 1 followed by 1 × 1 mL per kg bodyweight during days 2–7. During the subsequent maintenance period (weeks 4–8), subjects received 1 × 1 mL per kg bodyweight every second day. Blood samples were collected upon weeks 4, 6, and 8 to measure plasma D_2O enrichment.

Single-Trial Study

Prior to the 6-week intervention period, subjects participated in a single-trial study to evaluate acute myocellular signaling involved in transcriptional regulation of mitochondrial adaptations (**Figure 1**). Participants arrived at 7.30 am after overnight fasting. After 30 min supine rest, all subjects consumed a drink containing 20 g whey protein isolate (Whey 100 Extra Pure, Bodylab, Denmark). There is no consensus on use of feeding or fasting in studies, on acute effects but provision of a protein supplement is common in studies on protein synthesis. After consumption, participants assigned to BFRRE and HLRE completed a standardized warm-up (described in detail below) followed by a single bout of the prescribed exercise (i.e., BFRRE or HLRE – described in detail below). Participants assigned to CON performed all procedures of the single-trial study, except exercise. This was implemented to control for potential independent effects of e.g., the dietary standardization of the protocol, repeated biopsies, and/or diurnal rhythm, which has previously been justified by us to be important factors to control for (Vissing et al., 2005). Biopsies were collected from the vastus lateralis immediately (0 h) and (3 h) after completion of the single-trial study.

Arterial Occlusion Pressure

For participants assigned to BFRRE, arterial occlusion pressure (AOP) was determined before the single-trial study for determination of individualized cuff pressures. AOP was determined in a supine position after resting for 10 min, using a Doppler (Dopplex-D900, Huntleigh Healthcare Ltd., United Kingdom) as previously described (Sieljacks et al., 2018). BFRRE was conducted with a pressure corresponding to 50% of AOP, resulting in a mean (95% CI) exercise restriction pressure of 79 mmHg (74; 84 mmHg).

Training Intervention

One week after the single-trial study, participants initiated the 6-week intervention period. For BFRRE and HLRE groups, supervised training (see below) was conducted 3 days per week on non-consecutive days (Mondays, Wednesdays, Fridays). Before

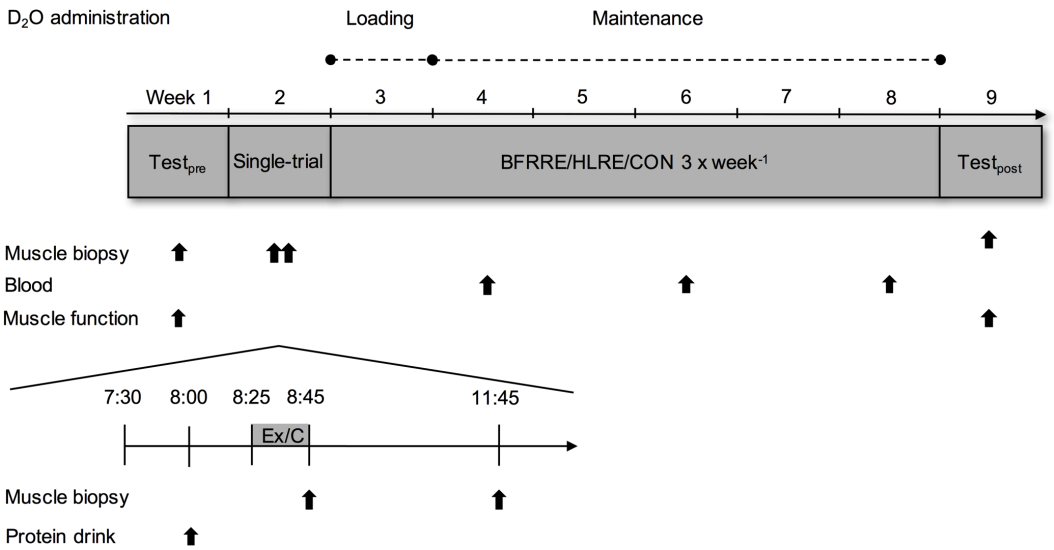

FIGURE 1 | Study overview. The total study length was 9 weeks and comprised a single-trial study and a long-term study. For the single-trial study, subjects arrived in the early morning after overnight fasting. After 30 min supine rest, a whey protein supplement was administered. After consumption, participants completed a BFRRE, HLRE, or CON single-bout session. Biopsies were harvested immediately after BFRRE/HLRE/CON (45 min after protein administration) and again 3 h after BFRRE/HLRE/CON (3 h and 45 min after protein administration). During the subsequent 6-week intervention period, BFRRE and HLRE groups conducted training 3 times per week. Throughout the 6-week intervention period, D_2O was orally administered to the participants with blood collected every second week to measure tracer enrichment. Biopsies and tests of exercise capacity were performed before and 4 days after the 6-week intervention period. BFRRE, blood flow restricted resistance exercise; CON, non-exercise control; HLRE, high-load resistance exercise; D_2O, deuterium oxide; Ex, exercise (i.e., HLRE or BFRRE); C, non-exercise control (i.e., rest).

each training session, subjects completed a light standardized warm-up consisting of 5 min of low intensity cycling (∼100 W) on a stationary bicycle ergometer (Monark Ergomedic 818E, Monark, Varberg, Sweden) followed by 1 × 5 knee-extension repetitions at a load corresponding to 50% of 1 repetition maximum (RM) and 1 × 5 knee-extension repetitions at a load corresponding to 70% of 1 RM in a knee-extension apparatus (TechnoGym selection-line, TechnoGym, Italy). For participants assigned to BFRRE, a 14 cm pneumatic cuff (Delfi Medical, Vancouver, Canada) was placed around the proximal portion of the thigh and inflated to 50% of AOP utilizing a digital tourniquet (A.T.S 2200TS, Zimmer Surgical Inc., OH, United States). This relative pressure was chosen, as it minimizes discomfort while proving equally effective compared to higher relative pressures (Loenneke et al., 2015; Counts et al., 2016). With the cuffs inflated, participants performed 4 sets of isolated knee-extensions to a state of volitional fatigue with a load corresponding to 30% of 1 RM interspersed by 30 s of inter-set recovery. During inter-set recovery, cuff pressure was maintained. Participants assigned to HLRE performed 4 sets of 12 knee-extension repetitions with a load corresponding to 70% of 1RM and 3 min of inter-set recovery.

Regardless of training regimen, a contraction duty cycle of 3 s (i.e., 1.5 s durations of concentric and eccentric phases) was controlled by auditory feedback from a metronome. The load was adjusted every second week by re-testing dynamic muscle strength. Furthermore, for HLRE, the exercise load was also progressively adjusted by a 5% increase once a subject could successfully perform 12 repetitions in all 4 sets. Conversely, if 10 repetitions could not be completed in the first set of a training session, the exercise load was immediately decreased by 5% for the subsequent sets.

Assessment of Dynamic Muscle Strength and Local Muscular Strength-Endurance Capacity

Maximal dynamic knee-extensor strength (1RM) was estimated using a 3RM-test as previously described (Brzycki, 1993). The 3RM test was conducted before the training intervention, every second week during the training intervention, and 4 days after completion of the intervention period. Local muscular strength-endurance capacity was evaluated before and 4 days after the intervention period, based on the corresponding 3RM-test. Accordingly, after completing the 3RM-test, subjects were given 10 min of rest and the load was adjusted to 30% of 1RM estimated on the corresponding visit. With a duty cycle of 3 s dictated by a metronome as described above, subjects then performed a single set of bilateral dynamic knee-extension exercise to concentric failure. The number of repetitions was used as an indication of local muscular strength-endurance capacity.

Muscle Biopsies

Muscle biopsies were collected at 0 and 3 h after the single-trial intervention, as well as before and after the long-term intervention using the Bergström needle technique (Bergstrom, 1975) under sterile conditions and local anesthesia (1% Lidocaine, Mylon Hospital, Norway). Muscle biopsies were

collected in a randomized manner with the pre, 0 h, and post-biopsies taken from one leg and the 3 h biopsy taken from the opposite leg to minimize repeated biopsy effects (Vissing et al., 2005). Importantly, all muscle biopsies were harvested from the same mid area of vastus lateralis, at the same depth in the muscle (approximately 1–2 cm), and a few centimeters distally from the distal site of occlusion.

After removal of visible fat and connective tissue, the muscle samples were allocated into separate tubes and preserved according to the respective analysis. In accordance, an aliquot of the biopsy (∼30 mg wet weight) was immediately submerged in an ice-cold relaxing buffer (BIOPS; in mmol L^{-1}: 2.77 CaK_2EGTA, 7.23 EGTA, 20 taurine, 6.56 $MgCl_2$, 5.77 Na_2ATP, 15 Na_2phosphocreatine, 0.5 dithiothreitol and 50 4-morpholineethanesulphonic acid; pH 7.1) and transported to the laboratory to be prepared for measures of mitochondrial respiratory function. Muscle samples for immunoblotting, enzyme activity, and FSR were frozen in liquid nitrogen and stored at −80°C until further analysis.

Tissue Preparation for Measurement of Mitochondrial Protein FSR

Mitochondrial protein FSR was determined over the 6-week intervention period from a sub-sample of the post-muscle biopsy. Body water enrichment was determined from plasma as previously described (Robinson et al., 2011; Scalzo et al., 2014; Konopka et al., 2017). To determine percentage of deuterium enriched alanine in a subcellular fraction of skeletal muscle enriched with mitochondria, we followed previously published standard operating procedures (Robinson et al., 2011; Drake et al., 2013; Scalzo et al., 2014; Konopka et al., 2017) that have been validated by both western blot and proteomic analysis of the fraction. Approximately 25–50 mg of skeletal muscle was homogenized in an isolation buffer containing 100 mM KCl, 40 mM Tris HCl, 10 mM Tris base, 5 mM MgCl2, 1 mM EDTA, and 1 mM ATP (pH 7.5), with phosphatase and protease inhibitors (HALT; Thermo Fisher Scientific, Rockford, IL, United States) with a bead homogenizer (Next Advance, Inc., Averill Park, NY, United States). After homogenization, the samples were centrifuged at 800 *g* for 10 min at 4°C. The resulting supernatant was further centrifuged at 9000 *g* for 30 min at 4°C. The resulting pellet was isolated and rinsed twice in a second isolation buffer (100 mM KCl, 10 mM Tris HCl, 10 mM Tris base, 1 mM MgCl2, 0.1 mM EDTA, 0.02 mM ATP, and 1.5% bovine serum albumin. pH 7.5) and once with distilled water. The pellet was resuspended in 250 μL of 1 M NaOH and placed on a heat block for 15 min at 50°C shaking at 900 rpm. The mitochondrial protein enriched fraction was then incubated in 6 N HCl for 24 h at 120°C for protein hydrolysis. The hydrolysates were ion exchanged, dried in a vacuum, and resuspended in 1 mL of molecular biology grade H_2O. Half of the suspended sample was derivatized by a 1 h incubation of 500 μL acetonitrile, 50 μL K_2HPO_4, pH 11, and 20 μL of pentafluorobenzyl bromide. Ethyl acetate was added and the organic layer was removed, dried under nitrogen gas, and reconstituted in 600 μL ethyl acetate for analysis on an Agilent 7890A GC coupled to an Agilent 5975C MS as previously described (Robinson et al., 2011; Scalzo et al., 2014; Konopka et al., 2017). The newly synthesized fraction (f) of mitochondrial proteins was calculated from the enrichment of alanine bound in muscle proteins over the entire labeling period, divided by the true precursor enrichment (p), using the average plasma D_2O enrichment over the period of measurement with MIDA adjustment (Busch et al., 2006).

Preparation of Permeabilized Muscle Fibers

The muscle sample was carefully dissected into two separated muscle fiber bundles (∼2.5 mg wet weight) in ice-cold BIOPS using the tip of two sharp forceps. The remaining muscle tissue was immediately frozen in liquid nitrogen for later analysis of citrate synthase activity. The muscle fiber bundles were chemically permeabilized by gentle agitation for 30 min in ice-cold BIOPS buffer containing saponin (50 μg mL^{-1}) as previously described (Anderson et al., 2009). Following permeabilization, the fiber bundles were washed twice by gentle agitation for 10 min in an ice-cold respiration medium (MiR05; in mmol L^{-1}: 110 sucrose, 60 K-lactobionate, 0.5 EGTA, 0.1% BSA, 3 $MgCl_2$, 20 taurine, 10 KH_2PO_4 and 20 HEPES; pH 7.1). Finally, fiber bundles were blotted dry and weighed on a microbalance (Mettler-Toledo, Greifensee, Switzerland).

High Resolution Respirometry

Mitochondrial respiratory function was measured in permeabilized muscle fibers by high-resolution respirometry using an Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria). Prior to each experiment, the polarographic oxygen sensors were calibrated in 2 mL MiR05. All experiments were conducted in duplicate at 37°C and in a hyperoxygenated environment to preclude potential O_2 diffusion limitations. We used a modified substrate/uncoupler/inhibitor-titration protocol from a previous study, designed to investigate various functional characteristics of the mitochondria (Jespersen et al., 2017). Accordingly, state 2 leak respiration supported by electron flow from complex I (GM) was measured by titration of glutamate (10 mmol L^{-1}) and malate (2 mmol L^{-1}). Subsequently, ADP (5 mmol L^{-1}) was added to yield information on coupled state 3 respiration supported by electron transfer from complex I (GM3). Integrity of the outer mitochondrial membrane was tested by cytochrome c (10 μmol L^{-1}) with an increase in respiration of >10% considered as a sign of damage leading to exclusion of data. Maximal coupled state 3 respiration supported by electron transfer from complex I and II (GMS3) was assessed by addition of succinate (10 mmol L^{-1}). State 4 respiration (4o) was then analyzed by addition of oligomycin (2 μg mL^{-1}). Following titration of oligomycin, maximal uncoupled respiration (E) was measured by stepwise titration of FCCP (0.5 μmol L^{-1}). Finally, residual oxygen consumption (ROX) was measured by addition of rotenone (0.5 μmol L^{-1}) and antimycin A (2.5 mmol L^{-1}). Individual titrations were performed with a minimum of 5 min intervals. If the signal was not stable, an additional 5 min was given. Inhibitor titrations (oligomycin, rotenone and antimycin A) were performed

with a minimum of 10 min to allow stable inhibition. Steady state respiratory rates were evaluated as average JO2 (oxygen consumption) over the stable period of the respiratory state using Datlab 6 software. Steady state respiratory rates (pmol s^{-1}) are expressed relative to milligram wet weight of muscle tissue. Data analysis was performed by a blinded investigator.

Citrate Synthase Activity

Frozen muscle tissue (∼25 mg wet weight) was freeze-dried for 24 h using a freeze-dryer (Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and homogenized for 2 min in 1.3 mL 0.3 mol L^{-1} K_2HPO_4 with 0.05% BSA/2 mg tissue using a tissuelyser II (Qiagen, Venlo, Netherlands). Fifteen minutes after addition of 10% Triton X-100 (10 μL/mL), insoluble materials were removed by centrifugation at 10,000 × *g* for 10 min at 4°C, and the supernatant stored for analysis of citrate synthase activity and total protein concentration. Total protein concentration was measured using a pierce 660 nm protein assay (Cat. 22660, Thermo Fisher Scientific, Rockford, IL, United States). Citrate synthase activity was measured spectrophotometrically using a citrate synthase activity assay kit (Cat. CS0720, Sigma-Aldrich, St. Louis, MO, United States). Briefly, homogenates were diluted 25 times in a reaction mix (Assay Buffer, 30 mM Acetyl CoA solution, 10 mM DTNB solution) and loaded on a 96 well half plate. After measuring background activity at wavelength 412 nm, 5 μL oxaloacetate (10 mmol L^{-1}) was added to each well to initiate the reaction. Light absorbance at 412 nm was measured every 10 s for 5 min at 37°C using a spectrophotometer (PHERAstar FS, BMG LABTECH, Ortenberg, Germany). Citrate synthase activity was calculated from the linear change in absorbance over time and normalized to total protein concentration.

Immunoblotting

After homogenization of freeze-dried muscle tissue, equal amounts of protein were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto polyvinylidene difluoride (PVDF) membranes as previously described (Rahbek et al., 2015). Protein concentration was assessed with a Bradford Assay (BioRad, CA, United States). Membranes were blocked for 2 h in 0.1% I-block (Applied Biosystems, CA, United States) TBST solution followed by overnight incubation with primary antibodies. All primary antibodies were purchased from Cell Signaling Technology and utilized as follows; p-ACC^{Ser79} (cat #3661, conc. 1:1000 in 5% BSA), p-$AMPK^{Thr172}$ (cat #2531, conc. 1:1000 in 5% BSA), p-$CaMKII^{Thr286}$ (cat #12716, conc. 1:1000 in 5% BSA) p-$CREB^{Ser133}$ (cat #9198, conc. 1:1000 in 5% BSA), p-p38 $MAPK^{Thr180/Tyr182}$ (cat #4511, 1:1000 in 5% BSA), p-$p53^{Ser15}$ (cat #9286, 1:1000 in 5% skim milk). Subsequently, membranes were incubated with secondary antibodies for 1 h with horseradish peroxidase-conjugated goat anti-rabbit (cat #6721 ABCAM, Cambridge, United Kingdom), except for p-p53, which was incubated with horseradish peroxidase-conjugated goat anti-mouse (cat #2055, Santa Cruz, TX, United States). A concentration of 1:5000 in 1% BSA was used for all secondary antibody solutions, except for p-AMPK and p-CaMKII antibody solutions where a concentration of 1:3000 in 1% BSA was used. Proteins were visualized by chemiluminescence (Thermo Fisher Scientific, MA, United States) and quantified with an UVP imaging system (UVP, CA, United States). Phospho-specific arbitrary protein intensity was normalized to total protein (i.e., total amount of protein loaded in the corresponding lanes) using Stain Free Technology as previously described (Gurtler et al., 2013). Results are presented as fold changes from pre.

Statistical Analyses

A one-way ANOVA was used to determine differences between groups in mitochondrial protein FSR. To compare total training volume and average time under ischemia per training session between BFRRE and HLRE, unpaired *t*-tests were utilized. Local muscular strength-endurance capacity, dynamic muscle strength, mitochondrial respiratory function, citrate synthase activity, and immunoblotting data, were analyzed by use of a linear mixed model with group, time, and time × group interaction as the factors of interest. Model validation included tests for equal standard deviations and examination of QQ-plots. We performed Pearson's correlation analysis on mitochondrial protein FSR and CS activity, and on mitochondrial protein FSR and measures of mitochondrial function with all groups pooled, the exercise groups pooled, and individual exercise groups. All statistical analyses were performed using STATA 15.0 (StataCorp, College Station, TX, United States) with $P < 0.05$ considered a statistically significant outcome. Graphic data are presented as means ± SD, whereas in text and in table data are presented as means with 95% CI.

RESULTS

Training Progression

Mean (95% CI) number of knee-extension repetitions performed in the first, second, third, and fourth set by BFRRE was 35.5 (31.8; 38.3), 11.6 (10.0; 13.2), 9.1 (7.9; 10.4), 8.4 (7.2; 9.5); by HLRE 11.4 (10.9; 11.8), 10.6 (9.9; 11.2), 9.6 (8.1; 10.42), 8.8 (8.1; 9.5). Average total volume of work (rep × load) performed was 59.7% higher for HLRE [57776.0 kg (49597.9; 65954.2 kg)] compared to BFRRE [36182.5 kg (31557.7; 40807.3 kg)] ($P < 0.01$). Average time under ischemia per training session (calculated as time under tension for HLRE and time under tension + inter-set recovery for BFRRE) was 135.8% longer for BFRRE [285.6 s (269.2; 302.0 s)] compared to HLRE [121.1 s (113.6; 128.6 s)] ($P < 0.01$).

Muscle Functional Capacity

There was an overall time × group interaction for local muscular strength-endurance capacity ($P < 0.01$) (**Figure 2A**). BFRRE increased strength-endurance capacity by an average of 28.3% (5.5; 51.0%) from pre to post-intervention ($P < 0.01$). There were no changes in strength-endurance capacity for HLRE and CON. There was a difference in strength-endurance capacity between CON and BFRRE post-intervention ($P < 0.05$). There were no differences in strength-endurance capacity between BFRRE and HLRE.

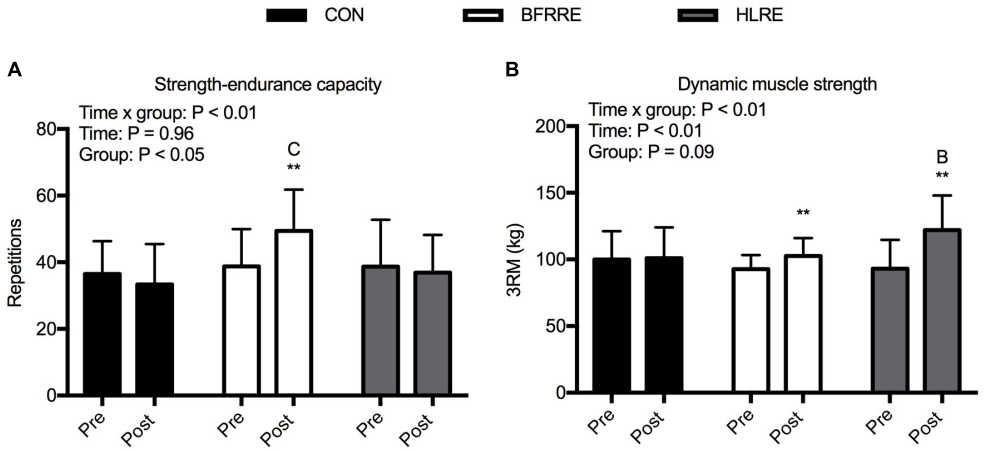

FIGURE 2 | Muscle functional capacity for CON, BFRRE, and HLRE groups. **(A)** strength-endurance capacity. **(B)** Dynamic muscle strength. ** denotes difference from pre-values within groups ($P < 0.01$), C denotes difference from CON at corresponding time point ($P < 0.05$), B denotes difference from BFRRE at corresponding time point ($P < 0.05$). Data are presented as means ± SD. Overall effects are designated in the upper left corner of each graph.

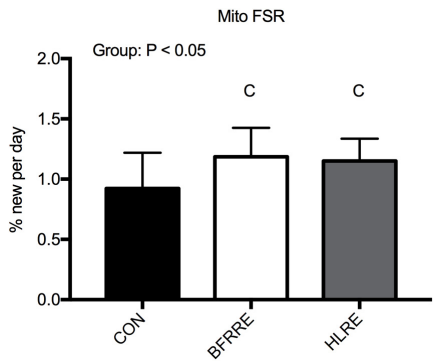

FIGURE 3 | Mitochondrial protein fractional synthesis rate (mito FSR) for CON, BFRRE, and HLRE groups. C denotes difference from CON ($P < 0.05$). Data are presented as means ± SD. Overall effect is designated in the upper left corner of the graph.

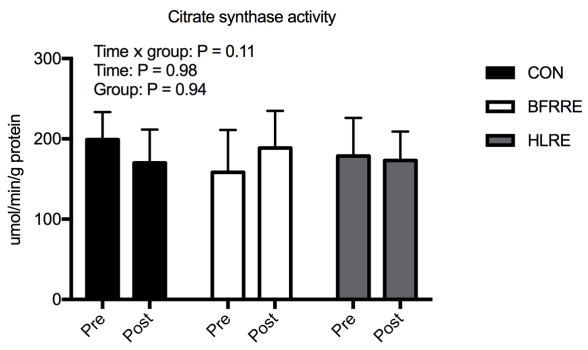

FIGURE 4 | Citrate synthase activity normalized to total protein for CON, BFRRE, and HLRE groups. Data are presented as means ± SD. Overall effects are designated in the upper left corner of the graph.

There was an overall time × group interaction for dynamic muscle strength ($P < 0.01$) (**Figure 2B**). BFRRE increased dynamic muscle strength by an average of 10.7% (4.4; 17.0%) from pre to post-intervention ($P < 0.01$). HLRE increased dynamic muscle strength by an average of 33.0% (17.5; 48.5%) from pre to post-intervention ($P < 0.01$). There were no changes in dynamic muscle strength for CON. There was a difference in dynamic muscle strength between BFRRE and HLRE post-intervention ($P < 0.05$).

Mitochondrial Protein Synthesis

Average body water enrichment remained stable through the labeling period [week 4: 0.80% (0.72; 0.87%)], week 6: 0.89% (0.79; 0.99%), week 8: 0.89% (0.76; 1.01%). Both resistance exercise training regimens increased ($P < 0.05$) mitochondrial protein FSR (%/day) compared to CON (**Figure 3**) [CON: 0.92%/day (0.71; 1.13%/day), HLRE: 1.15%/day (1.03; 1.27%/day), and BFRRE: 1.19%/day (1.02; 1.35%/day)]. There was no difference between BFRRE and HLRE.

Citrate Synthase Activity

For citrate synthase activity, there were no overall time × group interaction, time, or group effects **(Figure 4)**.

Mitochondrial Respiratory Function

Steady state respiratory rates per mg wet weight of muscle tissue are shown in **Figure 5**, with a representative graph of real-time oxygraph traces shown in **Figure 5A**. For state 2 leak respiration supported by electron flow from complex I (GM, **Figure 5B**), there was an overall effect of time ($P < 0.01$). Similarly, for state 3 respiration supported by electron flow from complex I (GM3, **Figure 5C**), there was an overall effect of time ($P < 0.05$), but the change over time was not statistically different between groups ($P = 0.12$). For state 3 respiration supported by convergent electron flow from complex I and II (GMS3, **Figure 5D**), there was an overall time × group interaction ($P < 0.01$). GMS3

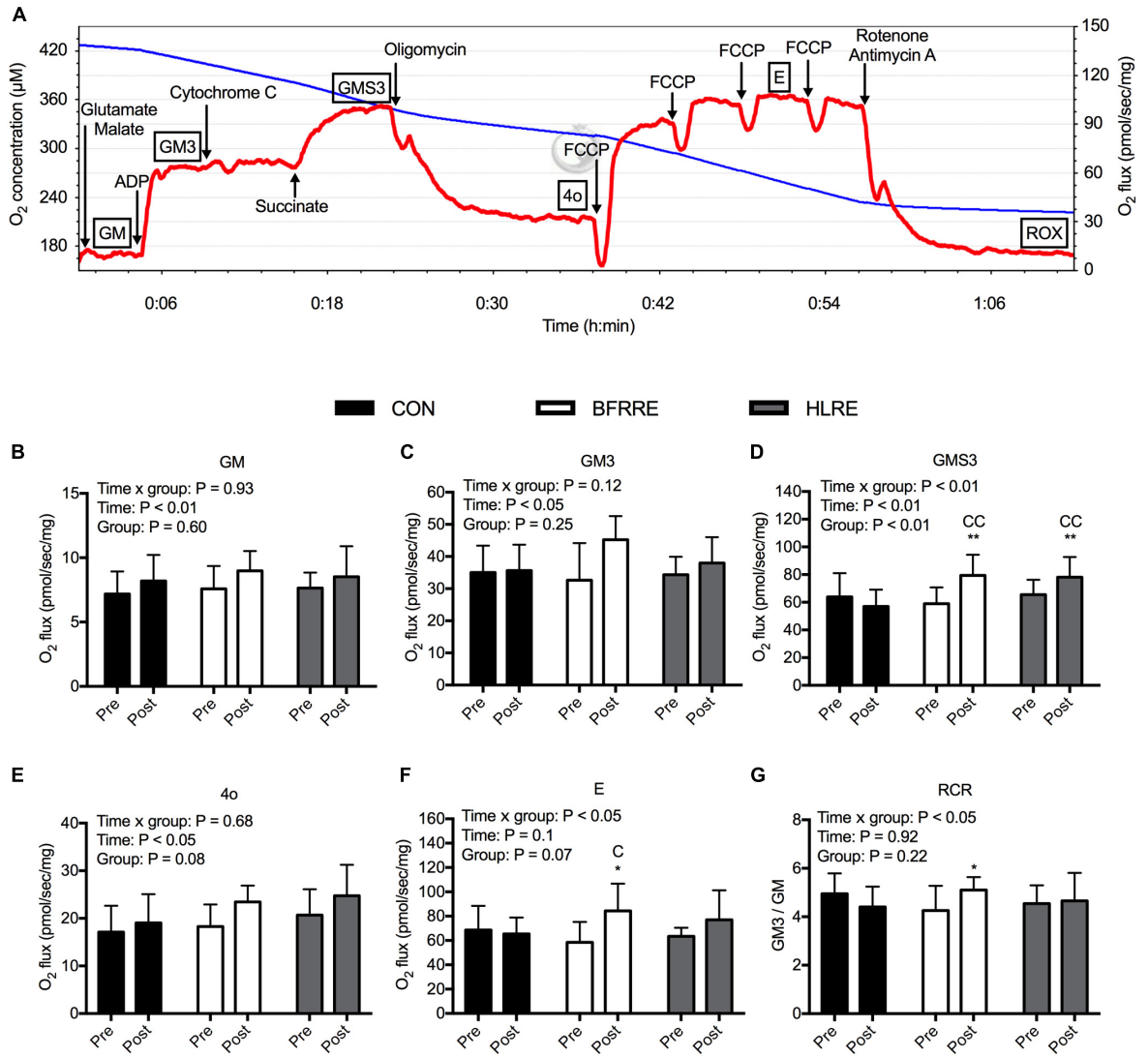

FIGURE 5 | Mitochondrial respiratory function in permeabilized muscle fibers for CON, BFRRE, and HLRE groups. **(A)** representative real-time oxygraph readout showing oxygen concentration in the chamber (blue line) and the calculated negative time derivative (oxygen flux) normalized to mg wet weight of muscle tissue (red line). Titrations are denoted with arrows and respiratory states are denoted with boxes. **(B)** state 2 respiration with glutamate and malate (GM). **(C)** state 3 respiration supported electron flow from complex I (GM3). **(D)** state 3 respiration supported by electron flow from complex I and II (GMS3). **(E)** state 4 respiration with oligomycin (4o). **(F)** maximal uncoupled respiration with FCCP (E). **(G)** respiratory control ratio (RCR) with complex I linked substrates. * denotes difference from pre-values within groups ($P < 0.05$), ** denotes difference from pre-values within groups ($P < 0.01$), C denotes difference from CON at corresponding time point ($P < 0.05$), CC denotes difference from CON at corresponding time point ($P < 0.01$). Data are presented as means ± SD. Overall effects are designated in the upper left corner of each graph.

increased by 37.6% (11.7; 63.5%) with BFRRE ($P < 0.01$) and 24.0% (7.1; 40.9%) with HLRE ($P < 0.01$). There were no changes in GMS3 for CON. After the intervention, GMS3 was higher with BFRRE ($P < 0.01$) and HLRE ($P < 0.01$) compared to CON. There were no differences in GMS3 between BFRRE and HLRE. For state 4 respiration with oligomycin (4o, **Figure 5E**), there was an overall effect of time ($P < 0.05$). We observed an overall time × group interaction for maximal uncoupled respiration (E, **Figure 5F**) ($P < 0.05$). E increased by 69.5% (18.6; 120.5%) with BFRRE ($P < 0.05$). There were no changes in E for CON and HLRE. After the intervention, E was higher with BFRRE ($P < 0.05$) compared to CON. There were no differences in E between BFRRE and HLRE. We observed an overall time × group interaction for the respiratory control ratio (RCR, **Figure 5G**) ($P < 0.05$). RCR increased by 19.8% (-5.7; 45.3%) with BFRRE ($P < 0.05$). There were no changes in RCR for CON and HLRE. There were no differences in RCR between BFRRE and HLRE.

In average, the fiber bundles weighed 2.48 mg (2.33; 2.62 mg). Six fiber bundles were excluded from data analysis based on the cytochrome c test. In these cases, data stem from one fiber bundle. In the cytochrome c test negative fiber bundles,

the addition of cytochrome c led to an increase in respiration of 0.36% (−0.73; 1.45%) confirming the integrity of the outer mitochondrial membrane.

Association Between Mitochondrial Protein FSR and Measures of Mitochondrial Function and Content

The correlation analyses are shown in **Supplementary Table 1**. Mitochondrial FSR was negatively correlated to the change in GMS3 specifically in the HLRE group ($P < 0.05$). Mitochondrial FSR was positively correlated to post-intervention GM3 and GMS3 when all groups were pooled ($P < 0.05$).

Acute Myocellular Signaling

Phosphorylation levels of signaling proteins involved in transcriptional regulation of mitochondrial adaptations and long-chain fatty acid uptake in the mitochondria are shown in **Figure 6** with representative immunoblots shown in **Figure 6A**. We observed an overall time × group interaction for p-p38 MAPK ($P < 0.05$) (**Figure 6D**). Phosphorylation of p38 MAPK increased 2.69-fold (1.36; 4.02-fold) ($P < 0.05$) and 3.25-fold (1.13; 5.37-fold) ($P < 0.05$) at 0 h with BFRRE and HLRE, respectively. There were no changes in p-p38 MAPK for CON. There was a difference in p-p38 MAPK between CON and BFRRE at 0 h ($P < 0.05$). There were no differences in p-p38 MAPK between BFRRE and HLRE. We observed an overall time × group interaction for p-ACC ($P < 0.05$) (**Figure 6C**). Phosphorylation of ACC increased by 2.17-fold (1.22; 3.12-fold) ($P < 0.01$) and 2.34-fold (1.75; 2.93-fold) ($P < 0.01$) at 0 h with BFRRE and HLRE, respectively. There were no changes in p-ACC for CON. There was a difference in p-ACC between CON and BFRRE at 0 h ($P < 0.01$). There were no differences in p-ACC between BFRRE and HLRE. For p-AMPK (**Figure 6B**), p-CaMKII (**Figure 6E**), p-CREB (**Figure 6F**), and p-p53 (**Figure 6G**), there were no overall time × group interaction, time, or group effects (for specific *P*-values see respective figures).

DISCUSSION

The present study is the first to investigate if low-load BFRRE stimulates skeletal muscle mitochondrial adaptations. We trained healthy young individuals by commonly recommended principles of practice of BFRRE or HLRE (American College of Sports Medicine [ACSM], 2009; Scott et al., 2015). By this approach, we show that BFRRE can stimulate long-term mitochondrial protein FSR and mitochondrial respiratory function in a manner similar to HLRE. These results support recent findings that resistance-type exercise can stimulate mitochondrial adaptations to support healthy skeletal muscle and whole-body metabolism (Wilkinson et al., 2008; Pesta et al., 2011; Donges et al., 2012; Porter et al., 2015; Holloway et al., 2018). Interestingly, BFRRE achieves similar mitochondrial adaptations at a markedly lower mechanical load, which have important implications in clinical situations that prohibit high loading, e.g., under post-surgery conditions, in patients suffering from arthritis, or in elderly frail individuals.

Mitochondrial Adaptations to Prolonged Resistance Training

In the present study, we used D_2O to assess long-term mitochondrial protein FSR. Both BFRRE and HLRE increased mitochondrial protein FSR, demonstrating that both exercise regimens stimulate long-term increases in mitochondrial biogenesis (Miller and Hamilton, 2012). Two previous studies used short-term primed continuous amino acid infusions to demonstrate acute increases in mitochondrial protein FSR in the recovery from unaccustomed single-trial HLRE (Wilkinson et al., 2008; Donges et al., 2012). In the study by Wilkinson et al. (2008) stimulation of mitochondrial protein FSR was attenuated when a similar single bout of HLRE was performed after 10 weeks of training habituation. In another study by Robinson et al., utilizing short-term primed continuous amino acid infusions, resting mitochondrial protein FSR was measured after overnight fast before and after a 12-week HLRE intervention (Robinson et al., 2017). In this study, HLRE significantly increased resting mitochondrial protein FSR in healthy older individuals but not in the young individuals. Our study extends these previous findings by measuring cumulative mitochondrial protein FSR by use of D2O, as previously accounted for (Simmons et al., 2016; Wilkinson et al., 2017). Most importantly, this approach allowed us to assess the practical benefits of the training protocols under free-living conditions so that all components of a 6-week training period (activity changes, changes in feeding status and diuernal rythm) were considered. By this approach, we show that both HLRE and BFRRE can increase cumulative mitochondrial protein FSR. However, we acknowledge that we cannot decipher the extent to which the synthetic response was primarily driven by large increases in the early phase of the training period, so this aspect warrants further investigation.

Both BFRRE and HLRE increased maximal coupled respiration supported by convergent electron flow from complex I and II (GMS3), which is suggestive of increased mitochondrial ATP-production capacity. This is in agreement with previous findings that HLRE can promote coupled respiration in permeabilized muscle fibers (Salvadego et al., 2013; Porter et al., 2015; Holloway et al., 2018). Two other studies have failed to demonstrate a similar effect of HLRE, but these studies measured respiration in isolated mitochondria (Irving et al., 2015; Robinson et al., 2017). The differences between studies can be attributed to disruption of mitochondrial morphology and organelle interactions inherent to studies on isolated mitochondria (Picard et al., 2011a,b).

Despite that BFRRE and HLRE increased mitochondrial biogenesis and mitochondrial respiratory function (GMS3), citrate synthase activity, a commonly used marker of mitochondrial content, did not change. While this is the first study to measure the effect of BFRRE on markers of mitochondrial content most previous studies have shown no changes or even decreases in response to HLRE (Groennebaek and Vissing, 2017). As previously discussed, it is not surprising

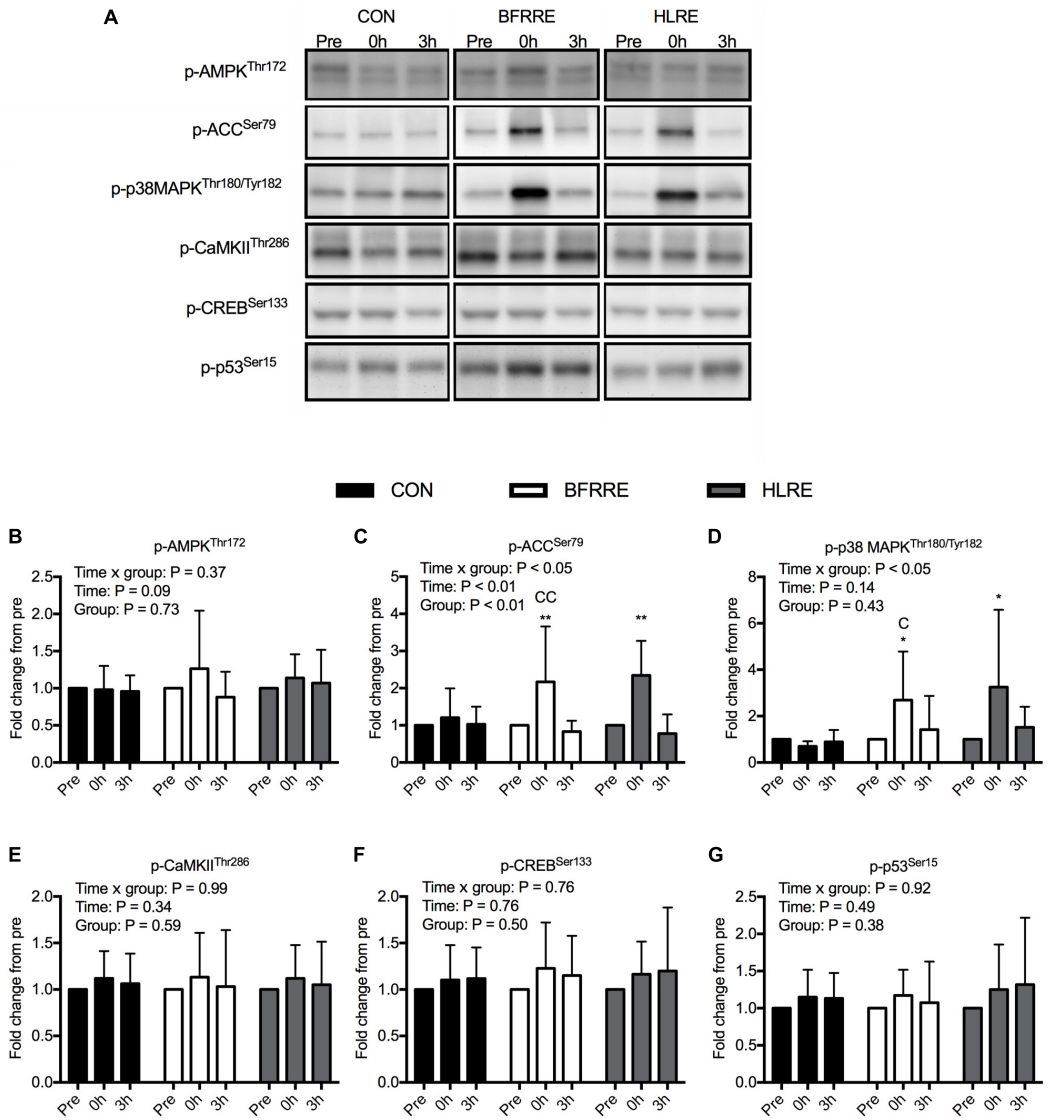

FIGURE 6 | Phosphorylation levels of signaling proteins involved in regulation of mitochondrial adaptations for CON, BFRRE, and HLRE groups. **(A)** representative immunoblots for all proteins at all conditions. **(B)** phosphorylated 5′ AMP-activated protein kinase (p-AMPK). **(C)** phosphorylated acetyl-CoA carboxylase (p-ACC). **(D)** phosphorylated p38 mitogen-activated protein kinase (p-p38 MAPK). **(E)** phosphorylated calcium/calmodulin-dependent protein kinase II (p-CaMKII). **(F)** phosphorylated cAMP response-element binding protein (p-CREB). **(G)** phosphorylated p53 (p-p53). * denotes difference from pre-values within groups ($P < 0.05$), ** denotes difference from pre-values within groups ($P < 0.01$), C denotes difference from CON at corresponding time point ($P < 0.05$), CC denotes difference from CON at corresponding time point ($P < 0.01$). Data are presented as mean fold changes ± SD. Overall effects are designated in the upper left corner of each graph.

that changes in mitochondrial biogenesis are not mirrored by similar changes in mitochondrial content (Miller and Hamilton, 2012; Miller et al., 2015). Increased mitochondrial biogenesis with maintained mitochondrial content suggests that mitochondrial breakdown also increased to increase overall protein turnover. The combination of increased mitochondrial biogenesis (assessed by mitochondrial protein synthesis), maintained mitochondrial content (assessed by citrate synthase) and increased rates of mitochondrial oxygen consumption (assessed by respirometry), provides strong evidence that BFRRE and HLRE stimulate mitochondrial remodeling to improve mitochondrial function (Drake et al., 2016; Ryu et al., 2016). However, we acknowledge that we do not provide direct evidence on activation of mitochondrial breakdown. Furthermore, we emphasize that while citrate synthase activity correlates with electron microscopy-derived measures of mitochondrial content (i.e., mitochondrial volume density) (Larsen et al., 2012; Meinild Lundby et al., 2017), exercise induced changes in citrate synthase activity and mitochondrial volume density do not always correlate well (Meinild Lundby et al., 2017; Granata et al., 2018). In accordance, the extent to which differentiated resistance exercise regimens facilitate changes in mitochondrial

content deserves further attention with the use of more rigorous techniques (e.g., electron microscopy).

Neither resistance training regimen improved coupled respiration supported solely by complex I substrates (GM3). This is in contrast to observations from a study on the effects of HLRE by Porter et al. (2015). This discrepancy may relate to different durations of the training interventions (6 weeks vs. 12 weeks), different exercise modes (single-limb vs. whole-body exercise), or a slightly different titration protocol. Interestingly, BFRRE increased the respiratory control ratio (RCR) with complex I substrates, calculated as the ratio between GM3 and GM, which suggests a tighter coupling between electron transfer and phosphorylation. No such effect was observed with HLRE, which is in accordance with Porter et al. (2015). On the other hand, Salvadego et al. (2013) observed a higher RCR in highly resistance trained subjects compared to sedentary controls (Salvadego et al., 2013). In the present study, BFRRE increased maximal uncoupled respiration (E), while HLRE did not. Porter et al. (2015) observed an increase in maximal uncoupled respiration in response to HLRE, but again, this discrepancy may be attributed to different training interventions and/or titration protocols. In summary, the functional mitochondrial adaptations to BFRRE at minimum match those of HLRE.

BFRRE training increased strength-endurance capacity when assessed at the same relative load while HLRE did not. HLRE training could have improved strength-endurance capacity had the test been conducted at the same absolute load. Since the chronic mitochondrial adaptations were similar between the two resistance exercise regimes, it is not clear if improvements in mitochondrial function and oxygen utilization mediated the differentiated adaptations in strength-endurance capacity with BFRRE and HLRE. It is possible that local oxygen consumption and/or capillary density differed, and that this may have contributed to differentiated adaptations in strength-endurance capacity with BFRRE and HLRE (Robbins et al., 2011), but for practical reasons we did not measure leg VO_2 and capillary density. Another likely explanation is that the BFRRE and HLRE have different neural innervation patterns. In accordance, BFRRE likely recruits smaller motor units until onset of fatigue development with accumulating work, whereas HLRE primarily recruits larger motor units throughout the work (Henneman, 1957; Farup et al., 2015). In contrast to strength-endurance capacity, both exercise regimens improved dynamic muscle strength. However, HLRE produced a more pronounced increase in dynamic muscle strength compared to BFRRE, which further support the concept of different neural innervation patterns.

Myocellular Signaling Underlying Mitochondrial Adaptations

In general, signaling responses to an acute bout of exercise were similar with both resistance exercise regimens.

It is known that acute perturbations in ATP turnover, calcium homeostasis, and/or ROS production stimulate mitochondrial adaptations (Hood et al., 2016). To obtain information on the type of stresses driving mitochondrial adaptations to BFRRE, we looked at responses to an acute exercise bout prior to the long-term training study. To account for ATP consumption and energy disruption associated with muscle contractions (Kjobsted et al., 2018) and/or ischemia (Kudo et al., 1995) we assessed AMPK activation through quantification of p-AMPK. We found that p-AMPK did not increase compared to CON with either training regimen. Previous studies measuring p-AMPK in response to resistance exercise have reported divergent results (Coffey et al., 2006; Wilkinson et al., 2008; Camera et al., 2010; Vissing et al., 2013). Discordance between these studies may relate to differences in dietary premise or timing of muscle biopsy sampling. In the current single-trial study, subjects were given a whey protein supplement to examine signaling changes under the common dietary approach of protein supplementation. It is possible that insulin and/or leucine may have promoted Akt-mediated phosphorylation of AMPKα Ser485/491 to antagonize phosphorylation of AMPKα Thr172 (Horman et al., 2006; Valentine et al., 2014; Kido et al., 2017). On the other hand, p-ACC, which is a downstream target of AMPK involved in signaling for fatty acid uptake into the mitochondria (McGee and Hargreaves, 2010), increased with both training regimens. p-ACC may therefore provide a read-out for AMPK activation (Richter and Ruderman, 2009), although other factors are also able to exert regulation on ACC (Brownsey et al., 2006). To control for independent effects of diet, diurnal rhythm, and repeated biopsies, we implemented a separate non-exercise control group. Since no changes occurred with CON, this indicates that metabolic perturbations imposed by resistance exercise can engage in signaling for regulation of long-chain fatty acid entry into the mitochondria (Abu-Elheiga et al., 2001). However, we do acknowledge that we did not accustom the participants to the exercise stimuli prior to the single-trial study. Consequently, signaling responses may not be representative of an exercise-familiarized state but may reflect an overall stress response typical of unaccustomed exercise (Benziane et al., 2008; Wilkinson et al., 2008). The signaling results should be interpreted with this in mind.

As for the ability of AMPK and CaMKII to engage in transcriptional regulation for mitochondrial adaptations, the observed lack of change in phosphorylation of AMPK and CaMKII suggests that the magnitude of perturbations in ATP turnover and calcium homeostasis were too modest to stimulate this (Chin, 2005; Kjobsted et al., 2018). This is supported by the simultaneous lack of phosphorylation changes in downstream transcription factors CREB and p53, both assumed to be involved in transcription of genes encoding mitochondrial proteins (Valsecchi et al., 2013; Bartlett et al., 2014). Opositely, p-p38 MAPK increased with both resistance exercise regimens. Increased phosphorylation of p38 MAPK could be ascribed to increased ROS production (Zhang et al., 2014), as result of occlusion-reperfusion during contraction and/or blood flow restriction, or to mechanical stress *per se* (Zhan et al., 2007).

In the current study, signaling responses for metabolic adaptations were similar between BFRRE and HLRE. In a previous study, it has been shown that acute low-intensity

cycling with blood flow restriction has little effect on metabolic signaling compared to traditional resistance training and endurance training (Smiles et al., 2017). Furthermore, it has been shown that application of external restriction of blood flow after sprint-interval training does not further accentuate phosphorylation of p-38 MAPK (Taylor et al., 2016). The effect of differentiated blood-flow restricted exercise regimens on signaling for metabolic adaptations deserves further attention with experimental designs that allow for more direct comparison.

Clinical Perspectives

The primary aim of our study was to determine if BFRRE could effectively promote mitochondrial adaptations. The rationale for this investigation was that low-load BFRRE imposes less force on the joints and may provide a more feasible alternative to HLRE for those recovering from surgery, in patients suffering from arthritis or in elderly frail individuals (Hughes et al., 2017). We have previously demonstrated that 6 weeks of low-load BFRRE and low-load free-flow resistance exercise performed to volitional fatigue were equally capable of producing pronounced muscle hypertrophy but with much less work and time expenditure per session required with BFRRE (Farup et al., 2015). In this context, the current findings are exciting. With regard to feasibility, it can be argued that even though BFRRE performed to fatigue is time-efficient, it can be experienced as uncomfortable in the unfamiliar state. However, in a previous study, we provided evidence that repeated bouts attenuate sensation of discomfort, similarly, to other types of unfamiliar exercise (Sieljacks et al., 2016). Moreover, all the subjects of the current study completed all training sessions without experiencing adverse events. Still, we acknowledge that similar studies need to be conducted in patient populations, to ultimately prove feasible and recommendable in the clinic.

CONCLUSION

The present study demonstrates that prolonged BFRRE as well as HLRE can stimulate long-term mitochondrial protein synthesis and increase mitochondrial respiratory function. Our results support that resistance exercise can promote important muscle metabolic adaptations and that the use of a low-load resistance exercise regimen is equally effective for achieving such adaptations. Future studies should focus on the use of BFRRE as an alternative to HLRE in clinical situations that prohibit high loading.

AUTHOR'S NOTE

The study was conducted at Section for Sports Science, Department of Public Health, Aarhus University.

AUTHOR CONTRIBUTIONS

TG, PS, ER, KH, BM, HB, FdP, and KV contributed to the conception and design. TG and KV wrote the first manuscript draft. All authors contributed to data acquisition and/or interpretation of data, critically revised the manuscript and provided intellectual contributions, and approved the final version of the manuscript submitted for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01796/full#supplementary-material

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Ischemic Preconditioning and Exercise Performance: An Ergogenic Aid for Whom?

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INTRODUCTION

Maneuvers of occlusion and reperfusion of the muscle blood flow aiming at improving exercise performance have been used since the 1950s with conflicting conclusions (Marocolo et al., 2016a). In the 80's decade, ischemic preconditioning (IPC) was defined as an intervention that consists of brief events of ischemia followed by reperfusion (Murry et al., 1986). It was described that a tissue, previously submitted to ischemic conditions, becomes more resistant to ischemia and its potential deleterious effects (Kocman et al., 2015). This remarkable clinical effect of IPC attracted sports scientists, and from 2000s many studies investigating the potency of IPC for enhancing exercise performance appeared massively in the scientific literature. Sport scientists attempted to demonstrate the beneficial effects of IPC on swimming (Marocolo et al., 2015; Ferreira et al., 2016), running (Sabino-Carvalho et al., 2017), cycling (Paradis-Deschênes et al., 2016), resistance (Marocolo et al., 2016b,c), and intermittent exercises (Marocolo et al., 2017) or general sports modalities (Incognito et al., 2016; Richard and Billaut, 2018). Nowadays, IPC is still studied for its ergogenic properties because it is simple, non-invasive, affordable and, thereby, readily applicable to exercise performance settings. While IPC can improve exercise performance, especially in endurance events, the mechanisms underlying its effects are unclear, as well as the robustness of the findings. Here we raise some methodological concerns about protocol design, data analysis, and interpretation, and discuss relevant positive effects and future directions for investigation.

METHODOLOGICAL CONCERNS

Although some studies have reported about positive effects of IPC on physiological responses and performance (see last section below), the reader must be aware that the scientific literature does not unanimously report beneficial effects. Rather, some papers reported null (Marocolo et al., 2017) or even negative effects (Paixao et al., 2014), and to date, the positive effects are highly contentious (Marocolo et al., 2016a). The below sections present some methodological aspects that must be addressed to move this field forward and find the optimal dose of IPC, if any.

DATA ANALYZES AND FITNESS OF THE SUBJECTS

Most studies investigating the effects of IPC on exercise performance have tested recreational/amateur subjects, with limited transfer to a higher level of competition in which ergogenic aids are highly relevant. Furthermore, most studies have carried out open-looped laboratory tests of unknown duration for the participant, and there is a paucity of studies dealing with self-paced exercise (e.g., field tests). Field tests are more similar to what athletes actually experience in training/competitions than in the laboratory. So, to move this research field forward,

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it is now critical to focus on the potential ergogenic effects of IPC on high-level fitness athletes tested in "real world scenario." The data analyzes of studies evaluating the efficacy of IPC are mainly based on statistical tests (p-value) or effect size (ES) comparisons to conclude about the presence or absence of "beneficial effects." Although there is a lively debate about the proper statistical approach that should be considered for exercise performance studies (Batterham and Hopkins, 2015; Welsh Knight and Knight, 2015), which is beyond the topic of this opinion article, the statistical results from approaches such as t-tests and ANOVA or ES, should be specifically interpreted. For example, a small ES found in elite athletes may be relevant, but a moderate/large ES in recreational subjects may not be. Therefore, until more robust and clear evidence demonstrates a beneficial effect in high-level athletes with at least a small ES, it is not advised to extrapolate the results observed in lower-end fitness subjects to high-level competitors. Analyzing the fitness level of subjects among almost 50 experimental studies that measured the effects of IPC on exercise performance in healthy subjects, one of them tested elite speed skaters (Richard and Billaut, 2018) while another evaluated elite cyclists (Paradis-Deschênes et al., 2018). Subjects evaluated in all other studies were amateur or recreational athletes, or even just healthy or sedentary (non-published data), even when the title or some part of the manuscript quoted "highly trained." In this sense, an interesting study (Foster et al., 2014) evaluating 12.8 km time trial in altitude found that running was faster when IPC was prior applied. However, their results were not statistical different and the ES was trivial (Marocolo et al., 2016a). Furthermore, another study testing swimmers (Jean-St-Michel et al., 2011) found benefits after IPC intervention, but again with trivial ES. Although the authors called "high-trained," actually they were not, since their 100 m swimming time of about 60 s are comparable to amateur swimmer. Generally, IPC effects on field and self-paced exercises present small magnitude for being considered effective. Therefore, we strongly encourage more research in real field performance targeting truly elite individuals.

IPC PROTOCOLS AND THE POTENTIAL PLACEBO EFFECT

Another issue that may have prevented scientists from reaching a consensus to date is the substantial variation among the IPC protocols (1–4 cycles of 2–5 min of ischemia) applied in exercise performance studies. While the potency of IPC may exist, it is possible that decades of research have not yet found the "correct protocol." While no effect on exercise performance have been demonstrated in several studies using standard protocols (e.g., 3 or 4 × 5-min occlusion/reperfusion; total ischemia of 15–20-min) (Marocolo et al., 2016a), a clinical study has shown that only 4 min of occlusion may be sufficient to reach a threshold for ischemic stimulus, regardless of the number of ischemic cycles (Ghosh et al., 2000). Since more ischemia cycles have not promoted greater enhancements in exercise performance (Cocking et al., 2018), new investigations should test the efficacy of shorter protocols. Intriguingly, shorter IPC protocols have not been examined, and if proven beneficial, these might offer a better option for athletes and coach staff due to obvious logistical reasons.

The placebo and/or nocebo effects are both methodological confounding factors in studies involving any potential ergogenic aid (Marocolo et al., 2015, 2017; Sabino-Carvalho et al., 2017). Specifically with IPC, a scientific debate raised questions about the real efficacy of IPC on performance. For instance, only 24% of the studies included in a systematic review, found beneficial effects of IPC on performance (or physiological variables) when a placebo control group was present (da Mota and Marocolo, 2016; Incognito et al., 2016). Vice versa, when a placebo group was not included in the analysis, the prevalence for positive effects increased (Incognito et al., 2016) (da Mota and Marocolo, 2016). Additionally, several studies found similar positive effects between low and high-pressure cuffing (i.e., SHAM and IPC) (Marocolo et al., 2016a,c; Sabino-Carvalho et al., 2017; Thompson et al., 2018). Thus, we could speculate that beyond potential placebo effects (Marocolo et al., 2015; Sabino-Carvalho et al., 2017), a bidirectional brain-body integration mechanism may promote physiological responses through mechanical-sensory receptors (Taylor et al., 2010; Cromwell and Panksepp, 2011).

Furthermore, it might be possible that the beneficial effect of IPC includes a lower perception of fatigue as a potential mechanism. Indeed prior experiments have been suggested that IPC can potentially desensitize group III and IV nerve endings to metabolite accumulation. It is stated that type III and IV nerve endings exert contribution to the cardiovascular regulation during exercise (Amann et al., 2011), which could direct contribute to changes in performance. These types of nerve (III and IV) modulate the sympathetic tone based on mechanical and metabolic conditions on the working muscle, acting type III mainly as mechano- and IV as metabo-receptors (Nobrega et al., 2014). When the end part of this nerve is stimulated, increases in sympathetic tone, regulating hemodynamic parameters such as heart rate and cardiac contractility (Crisafulli et al., 2011; Marongiu et al., 2013).

It was found increases in handgrip performance after IPC, without changes in blood flow, conductance and muscular deoxygenation, with differences in the slowing of contraction and relaxation throughout the exercise (Barbosa et al., 2015). This finding suggests that IPC could affect some neural pathway. Corroborating their results, another study (Mulliri et al., 2016) investigated the effect of IPC on the hemodynamics during metaboreflex recruitment and found reductions in mean arterial pressure response and impairs venous return, possible through increases in nitric oxide production. Although the second study did not support the hypothesis that IPC improves performance in exercise with limited muscle mass, they showed that IPC affects hemodynamics. Future research should better clarify the relation between IPC and the role of type III and IV nerve endings.

POSITIVE EFFECTS OF IPC

This opinion paper has presented current scientific pitfalls to data interpretation about the efficacy and applicability of IPC.

Nonetheless, we recognized that IPC has been shown to elicit beneficial, ergogenic adaptations conducive to enhanced physical capacity. The recent meta-analysis performed by Salvador et al. (2016) demonstrated a real impact on sports performances. When all types of performances were combined, IPC yielded a "small" beneficial (ES 0.43) effect with no chance of observing a negative impact (ES range 0.28–0.51). The most robust beneficial impact was reported for "aerobic" (>90 sec) performances with a "moderate" (ES 0.51) impact. Results for performances of shorter duration were not convincing (Marocolo et al., 2016b; Salvador et al., 2016). A detailed look at the scientific literature reveals relevant findings for athletes. For example, aerobic power output measured during an incremental cycle test to volitional fatigue went up by 3% ($p<0.01$) from 366W to 372W in athletes (de Groot et al., 2010). This finding is in line with performances of runners reported running a 5-km time trial 34 s faster ($p<0.05$) after using IPC (Bailey et al., 2012). IPC has been reported to improve maximal performance in various exercise modes when the oxidative system is fully taxed (de Groot et al., 2010; Bailey et al., 2012; Kjeld et al., 2014). Along this line, some evidence exists showing that IPC can, in some cases, enhance performance during the hypoxic insult. A study reported greater power output and faster time to complete a 5-km time trial in cyclists at 2,500-m simulated altitude (Paradis-Deschênes et al., 2018). These enhanced aerobic performances may be related to acute molecular and vascular adaptations that promote local vasodilation, enhance blood flow, and ultimately improve O_2 delivery and utilization (Tapuria et al., 2008; Beaven et al., 2012). However, trends in muscle oxygenation are not always clear after IPC. Studies have reported attenuated (Kido et al., 2015; Patterson et al., 2015), accentuated (Barbosa et al., 2015; Paradis-Deschênes et al., 2016) and accelerated dynamics (Kido et al., 2015; Tanaka et al., 2016), which complicates the understanding of the IPC-induced physiological mechanisms.

Although meta-analyses do not favor IPC in enhancing shorter performances see Salvador et al., 2016, some studies still reported interesting findings for the "anaerobic" athlete. The muscular force developed during repeated maximal isokinetic contractions was enhanced in strength-trained athletes (Paradis-Deschênes et al., 2016) and swimmers could produce the fastest times during 50-m (1.2%, $p < 0.05$, Lisbôa et al., 2017) and 100-m (1.1%, $p < 0.01$, Jean-St-Michel et al., 2011) sprints after using IPC compared to sham occlusions. These data are certainly of practical importance during competitions. However, other studies could not report any superior performances after IPC (Patterson et al., 2015), which demonstrates that the context of the application, the protocol and, probably, the type of athletes influence the outcomes of this technique.

FUTURE DIRECTIONS AND CONCLUSIONS

This opinion piece is aimed at raising awareness in athletes and coaches, and to call upon researchers to urgently address current experimental pitfalls that obscure our understanding. We believe that future studies should test shorter protocols (e.g., 2 × 2–3 min occlusion/reperfusion), which are more time-efficient (e.g., 8–12 min vs. 40 min) and more easily inserted in real-world settings of athletes/competitions if positive and meaningful findings are confirmed. Also, testing treatments controlled by different cuffing pressures (i.e., SHAM, IPC, and no cuff—control) should assess the effect of IPC on higher fitness subjects (i.e., elite athletes). Then only, we may be able to draw robust conclusions as to whether IPC is suitable for recreational practitioners and/or elite athletes.

AUTHOR CONTRIBUTIONS

MM and GdM made substantial contributions to the conception, design, and drafting of the work, as well as the analysis and interpretation of data for the work. MM, FB, and GdM revised it critically for important intellectual content. MM, FB, and Gdm provided approval for publication of the content. MM, FB, and GdM agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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12-Week Exercise Training, Independent of the Type of Exercise, Attenuates Endothelial Ischaemia-Reperfusion Injury in Heart Failure Patients

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Introduction: Reperfusion is required to salvage ischaemic tissue, but also causes further damage (i.e., ischaemia/reperfusion-injury). Heart failure patients reveal exaggerated ischaemia/reperfusion-injury, whilst traditional ischaemic preconditioning cannot prevent ischaemia/reperfusion-injury. Exercise training may be a more powerful preconditioning stimulus, especially high-intensity interval training given the similarities with ischaemic preconditioning. Therefore, we examined the impact of 12-week continuous training vs. high-intensity interval training on brachial artery endothelial ischaemia/reperfusion-injury in heart failure patients New York Heart Association-class II-III.

Methods: Twenty heart failure patients (male:female 19:1, 64 ± 8 years, ejection fraction 38 ± 6%) were allocated to 12-weeks of high-intensity interval training (10*1-min 90% maximal workload – 2.5-min 30% maximal workload) or continuous training (30-min 60–75% maximal workload). Before and after the intervention, we measured brachial artery endothelial function with flow-mediated dilation (FMD) before and after ischaemia/reperfusion (5-min ischemic exercise, 15-min reperfusion).

Results: Ischaemia/reperfusion caused a significant decline in FMD (continuous training (n = 10): 5.2 ± 2.5 to 3.4 ± 1.6%, high-intensity interval training (n = 10): 5.3 ± 2.6 to 3.5 ± 1.6%, P = 0.01), which was not different between groups ($P > 0.05$). Training improved maximal workload and fitness ($P < 0.05$), with no differences between groups ($P > 0.05$). Exercise training did not alter FMD ($P > 0.05$), whilst ischaemia/reperfusion did not impair FMD after exercise training (continuous training: 4.8 ± 3.0 to 4.2 ± 2.3%, high-intensity interval training: 4.7 ± 2.5 to 3.8 ± 2.3%, $P > 0.05$). No changes were found in FMD before or after ischaemia/reperfusion after 12-weeks in controls (n = 9).

Conclusion: We found that 12-week exercise training in heart failure patients mitigated endothelial ischaemia-reperfusion injury, an effect independent of the type of exercise. These changes may contribute to the cardioprotective effects of exercise training, whilst our findings highlight the potency of exercise as a preconditioning stimulus.

Keywords: exercise training, preconditioning, physical fitness, cardiovascular function, flow-mediated dilation

INTRODUCTION

The prevalence of heart failure (HF) is increasing, and is characterized by a low 5-year survival of 35–55% (Bleumink et al., 2004). One potential reason for this poor prognosis may relate to ischaemia-reperfusion (IR)-injury. Although reperfusion is a common and effective strategy to restore blood flow to ischaemic (cardiac) tissue (Piccolo et al., 2015), this paradoxically causes significant additional damage (i.e., IR-injury) to the endothelium (Yellon and Hausenloy, 2007). Attenuating the deleterious effects of IR is therefore of utmost importance to further improve outcomes after myocardial infarction. Previous work in rats (Murray et al., 2006) and recently work from our group in humans (Seeger et al., 2016) revealed that HF is associated with exaggerated endothelial IR-injury. This highlights the need to explore strategies to attenuate endothelial IR-injury in HF patients.

Ischemic preconditioning (IPC) (i.e., short repetitive episodes of non-injurious ischemia and reperfusion) represents a potent strategy to reduce the severity of endothelium IR-injury (Murry et al., 1986). However, clinical trials using IPC have revealed somewhat disappointing results (Heusch, 2013), which may relate to the interaction between cardiovascular disease and the efficacy of IPC (van den Munckhof et al., 2013; Ferdinandy et al., 2014). Indeed, preclinical studies (Miki et al., 2000; Ghosh et al., 2001; Andersen et al., 2013) and *in vivo* work in humans (Seeger et al., 2016) revealed that HF is associated with an attenuated ability of IPC to prevent (endothelial) IR-injury. Interestingly, previous work in animals demonstrated that exercise training, in line with IPC, results in myocardial adaptation that allows greater recovery of cardiac function after cardiac ischaemia (Bowles et al., 1992). These cardioprotective effects against IR injury seem present after both moderate- and high-intensity training in rats (Lennon et al., 2004). Although the exact mechanisms are currently incompletely understood, and may even differ from those related to IPC, exercise may represent an alternative preconditioning stimulus that may contribute to protection against IR-injury (Thijssen et al., 2018).

Regular exercise training improves the risk against cardiovascular events (Mora et al., 2007; Green et al., 2017). Animal studies revealed that exercise training restores the attenuated efficacy of IPC in aged rat hearts (Abete et al., 2000; Wang et al., 2014). Similarly, we recently reported that lifelong exercise training is associated with increased tolerance against endothelial IR-injury (Maessen et al., 2017). Accordingly, exercise training may also attenuate endothelial IR-injury in patients with HF. In addition, the type of exercise may impact the benefits of exercise training. Michelsen et al. (2012) found that interval exercise (which shows similarities with ischaemic preconditioning in mediating repeated, short bouts of local ischaemia) induces immediate cardioprotection. Moreover, we found that a single bout of interval exercise, but not endurance exercise, protected against endothelial IR-injury in healthy young men (Seeger et al., 2015).

In the present study, we examined the effect of 12-weeks of CT or HIT on the magnitude of decline in endothelial IR-injury in HF patients. In line with recent observations (Maessen et al., 2017; Thijssen et al., 2018), we expect that exercise training will attenuate the (exaggerated) decline in endothelial function in response to IR-injury in HF patients. Moreover, based on the acute preconditioning effects of interval exercise (Michelsen et al., 2012; Seeger et al., 2015), we expect that HIT shows superior effects compared to traditional CT.

MATERIALS AND METHODS

Subjects

A total of 29 patients (65 $\pm$ 8 years) diagnosed with HF [NYHA class II-III, history of left ventricular ejection fraction (LVEF) $\leq$ 45%] were included in our study for final analysis. Inclusion took place through advertisement, and via the Department of Cardiology of the Radboud University Medical Center and the Canisius-Wilhelmina Hospital (Nijmegen, Netherlands). We excluded patients who developed HF due to congenital heart disease and/or valve pathology. We excluded the following individuals who present with: diabetes mellitus (type 1 or 2), hypercholesterolemia (total cholesterol $>$ 6.5 mmol/L), severe renal failure (glomerular filtration rate $<$ 30 mL/min/1.73 m^2), exercise-induced ischemia (i.e., ECG abnormalities suggestive for ischemia on maximal exercise testing), severe co-morbidities (e.g., COPD GOLD $\geq$ 3), pathology that restricts patients from participation to exercise (e.g., orthopedic/neurological disorders interfering with movement), pre-menopausal women or women on hormone replacement therapy, and subjects with contra-indications for maximal exercise testing (Fletcher et al., 2013). All individuals were in a stable situation, meaning that clinical and pharmacological status has not changed $>$ 3 months prior to participation. We received ethical approval from the local Medical Ethical Committee (CMO region Arnhem–Nijmegen; Geert Grooteplein 10, 6525 GA Nijmegen, Netherlands), whilst our trial is registered in the Dutch Trial Register (NTR3671). Written informed consent was obtained before participation in this study.

Experimental Protocol

After inclusion into our study, subjects were allocated to 12-weeks moderate-intensity CT or HIT. To control for potential changes across time, measurements were performed before and after a 12-week control period in nine HF patients unable to participate (due to transportation or time-constraints). Before and after the intervention, we examined physical fitness (using a maximal incremental cycling test) and vascular ultrasound to examine brachial artery flow-mediated dilation (FMD) before and after an IR-protocol as a surrogate for IR-injury (Kharbanda et al., 2002; Loukogeorgakis et al., 2005, 2006). Although blinding of participants was not possible, blinding of the observer during FMD analysis was applied for the allocation of the group and timing of the test. The study was originally set-up to examine the impact of both types of exercise training on clinical outcome measures (i.e., physical fitness, quality of life) and cardiac and vascular function/structure, which is published elsewhere (Benda et al., 2015a). The changes in brachial artery endothelial function after IR represented a secondary outcome measure, and were not part of the original analysis.

Measurements

Subject Characteristics

Height, weight (Seca 888 Scale, Seca, Hamburg, Germany), BMI, body fat percentage (Durnin and Womersley, 1974), and waist and hip circumference were determined before and after the intervention. Heart rate and blood pressure were measured manually (WelchAllyn, Maxi-Stabil 3, Skaneateles Falls, NY, United States), whilst an electrocardiogram was used to assess cardiac rhythm. A venous blood sample was used to assess levels of fasted glucose and cholesterol.

Physical Fitness

Subjects performed an incremental maximal cycling test (Ergoline, Ergoselect 200k, Bitz, Germany). Subjects were instructed to pedal at a constant speed (>60 rpm) whilst workload was increased 10–15 Watt/min (dependent on sex, age, height, and previous results). Continuous breath-by-breath gas analysis was used to examine changes in oxygen uptake (LabManager V5.32.0). Peak oxygen uptake (VO_{2peak}) was defined as the highest 30-s oxygen uptake during the test. We adhered to recent guidelines for the termination of the exercise test (Fletcher et al., 2013).

Endothelial Function

Before each experiment, participants refrained from food ingestion $\geq$ 6 h, caffeine and products with high levels of vitamin C $\geq$ 18 h, and from strenuous physical activity $\geq$ 24 h. Subjects were tested at the same time of day to prevent diurnal variation in FMD response. All measurements were performed in a temperature-controlled room (22.5°C) and using expert-consensus guidelines of FMD (Thijssen et al., 2011; van Mil et al., 2016). Subjects were instructed to continue medication, but to refrain from diuretics the day of testing for practical reasons. Subjects rested in a supine position with the right arm extended and immobilized, supported at an angle of ∼80° abduction from the torso. For the assessment of FMD, a rapid inflation/deflation pneumatic cuff was placed distal to the olecranon process to provide an ischaemic stimulus distal from the brachial artery to provoke vasodilation. A 10-MHz (T3000, Terason, Aloka, United Kingdom) multi-frequency linear array probe attached to a high-resolution ultrasound machine was used to perform imaging. Ultrasound parameters were set to optimize longitudinal B-mode images of the lumen/arterial wall interface. A continuous Doppler velocity assessment was obtained simultaneously, and data were collected using the lowest possible insonation angle (always < 60°), which did not vary during each study (Thijssen et al., 2011). After a resting period of > 15-min, 1-min of baseline recording of the arterial diameter and velocity was performed. Subsequently, the occlusion cuff was inflated to 220 mmHg for 5-min. The arterial diameter and velocity recordings were restarted at least 30 s before cuff deflation and continued for at least 3 min after deflation. Peak arterial diameter and flow, and the time to reach this peak after cuff deflation, were recorded. Analysis of the brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias. Following cuff deflation, peak diameter was automatically detected according to an algorithm as described in detail elsewhere (Black et al., 2009). Within-subject reproducibility of the FMD using this semi-automated software is 6.7–10.5% (coefficient of variation) (Thijssen et al., 2009).

Endothelial Ischaemia-Reperfusion

Ischaemia-reperfusion was induced by a 5-min ischaemic handgrip exercise stimulus followed by 15-min of reperfusion. Local ischaemia during handgrip exercise (rhythmic handgrip exercise at 30% of maximum handgrip strength, 1 s contraction followed by 1 s rest) was induced with upper arm cuff inflation to 220 mmHg. This ischaemic handgrip protocol leads to a (near) maximal ischaemic stimulus and peak reactive hyperaemia (Naylor et al., 2005). The transient decrease in FMD is assumed to reflect IR-induced endothelial dysfunction, a finding supported by studies that successfully mitigated this decline in FMD by well-established pharmacological (i.e., statins) and physical (i.e., ischaemic preconditioning; Kharbanda et al., 2001; van den Munckhof et al., 2013) interventions that protect against IR. Furthermore, brachial artery FMD correlates with coronary artery endothelial function in humans (Takase et al., 1998), and predicts cardiovascular events in asymptomatic subjects and in those with established cardiovascular diseases (Inaba et al., 2010; Ras et al., 2013). This model, therefore, is a frequently used and surrogate endpoint for IR-injury (Kharbanda et al., 2001; van den Munckhof et al., 2013).

Exercise Training

Supervised exercise training was performed in a rehabilitation/hospital setting (twice a week). All missed exercise sessions were replaced to ensure a 100% compliance. Warm-up consisted of 10-min at 40% of maximal workload (W_{max}) and concluded with a 5-min cool-down at 30% W_{max}. Workload was gradually increased across the training period. CT consisted of 30-min at 60–75% W_{max}, aiming at a Borg score of 12–14 (Piepoli et al., 2011). HIT consisted of 10 periods of

intervals of 1-min at 90% W_{max} followed by 2.5-min at 30% W_{max}, aiming at a Borg score of 15–17 during the high-intensity intervals. Control subjects were instructed not to alter their daily physical activities. A frequency of twice a week was adopted to match the exercise training regimes typically adopted in cardiac rehabilitation to ensure that the observations from our study can be more easily translated to daily route in HF management.

Statistical Analysis

We have made a pre-study sample size estimation based on previous studies examining the difference in effect between CT and HIT. No previous study examined the impact of exercise training on endothelial IR. We therefore based our estimations on previous studies examining the impact of exercise training on vascular function measured using the FMD. Some studies suggest n = 2–3 per group is sufficient (Wisloff et al., 2007; Fu et al., 2013), whilst data from others suggest several thousand subjects must be recruited to detect differences between CT and HIT (Iellamo et al., 2013). We rationalized that n = 10–20 will provide (clinically) meaningful insight into the effect of exercise training. Therefore, we aimed for n = 20 for both exercise training groups (and n = 10 in the control group).

Data was analyzed using SPSS Statistics 20.0 (IBM Corp., Armonk, NY, United States). Parameters were checked for normality using a Kolmogorov–Smirnov test. When data was not normally distributed, a non-parametric alternative was used or natural logarithmic data transformation was applied. Categorical and nominal parameters were compared with a Chi-Square test. Baseline characteristics of the groups were compared with a one-way ANOVA or Kruskal–Wallis test when data was not normally distributed. Data are presented as mean ± standard deviation (SD), unless stated otherwise. Significance level was set at $P < 0.05$.

To examine the impact of exercise training ("time": pre vs. post) and the type of exercise ("type": CT vs. HIT) on the change in FMD after IR ("IR": baseline vs. post-IR), we adopted a linear mixed model analysis. To control for the potential impact of within- and between-subject differences in baseline diameter on FMD (Atkinson and Batterham, 2013), we used logarithmically transformed diameter data included baseline arterial diameter as a covariate within the linear mixed model analysis. For aim 1, FMD was analyzed with random factor subject and 2 fixed factors: time (pre vs. post) and IR (baseline FMD vs. post-IR FMD). When a significant interaction-effect was found, we adopted *post hoc* analysis to identify differences. To examine whether the type of exercise impacted the effect of exercise training (i.e., aim 2), we repeated this analysis with the addition of "type" (CT vs. HIT) as a fixed factor.

RESULTS

Out of the 59 individuals who were screened for this study, 15 HF patients did not meet the inclusion criteria and 11 patients declined participation (10 due to time constraints, 1 due to illness). Twenty-four individuals were randomly assigned to HIT or CT, whilst 9 HF patients were included as controls (non-randomized). No drop-outs were observed in the control group. A total of 4 drop-outs were present (71 ± 2 years; male:female 3:1; NYHA class II:III 3:1). In both exercise training groups 1 person dropped out because clinical progression and 1 due to musculoskeletal complaints. Except for sex (i.e., more females in the control group), we found no differences between groups in body characteristics (e.g., age, BMI) or clinical status (e.g., NYHA-class, etiology, blood pressure, LVEF, physical fitness) (**Table 1**).

Exercise Training

Exercise intensity of CT was 66 ± 5% of maximal workload. The intervals during HIT were performed at 102 ± 7% of maximal workload ($P < 0.001$). Relating average exercise intensity across the entire exercise bout to individual peak heart rates, we found that CT was performed at 81 ± 7% and HIT at 83 ± 9% of maximum HR ($P = 0.70$). Subjective exercise intensity measured using Borg-scores revealed no difference between CT and HIT (13 ± 1 and 14 ± 1, respectively, $P = 0.27$). We found no significant increase in physical fitness after training when presented as VO_{2peak}, whilst a significant increase was found when presented as percentage of the predicted VO_{2peak} (**Table 2**). For both parameters, no differences were found between groups (both $P = 0.08$, **Table 2**). Maximum workload improved after CT and HIT (both $P < 0.001$), whilst these changes also did not significantly differ between groups ($P = 0.07$, **Table 2**).

Endothelial Ischaemia-Reperfusion Injury

Prior to training, brachial FMD significantly declined in response to IR (**Table 3**). Exercise training in HF patients did not alter brachial artery FMD (**Table 3** and **Figure 1**). *Post hoc* analysis revealed that after 12-week exercise training, IR did not change FMD (**Figure 1**).

Statistical analysis revealed no significant differences in baseline FMD or in the magnitude of decline in FMD in response IR between the CT and HIT groups (**Table 2**). Furthermore, no significant differences were found for the impact of the type of training (i.e., CT vs. HIT) on baseline FMD ("time*type"-interaction, $P = 0.81$) or for the magnitude of decline in FMD post-IR ("time*type*IR"-interaction, $P = 0.99$).

Control Group

The control group showed no change in VO_{2peak} (1.36 ± 0.56 vs. 1.39 ± 0.60 L, $P = 0.50$) or predicted VO_{2peak} (81 ± 22 vs. 82 ± 21%, $P = 0.74$) over 12 weeks without additional training. We found no change in baseline brachial artery FMD or in the magnitude of decline in brachial artery FMD after IR (**Table 4**).

DISCUSSION

This study is the first study in humans to examine whether regular exercise training affects the exaggerated endothelial IR-injury observed in HF patients. Our study provides the following observations. First, we found that 12-weeks of exercise training in HF patients attenuated the magnitude of endothelial IR-injury in HF patients, whereas these beneficial effects are not accompanied by an improvement in baseline endothelial function. Second,

TABLE 1 | Subject characteristics and cardiovascular medication.

	CT (*n* = 10)	HIT (*n* = 10)	Control (*n* = 9)	*P*-value
Age (years)	64 ± 8	63 ± 8	67 ± 7	0.57
Sex (male:female)	10:0*	9:1	5:4	0.028
Body mass index (kg/m^2)	28.9 ± 4.7	28.1 ± 7.5	25.4 ± 2.7	0.24
NYHA class (II:III)	8:2	8:2	8:1	0.84
Etiology (Isch:Non-isch)	8:2	7:3	5:4	0.51
Systolic blood pressure (mmHg)	132 ± 23	132 ± 18	130 ± 25	0.98
Diastolic blood pressure (mmHg)	83 ± 11	79 ± 10	78 ± 14	0.48
VO_{2peak}† (mL/min/kg)	21.0 ± 3.4	19.1 ± 4.1	17.4 ± 5.8	0.26
VO_{2peak}† (% of predicted VO_{2peak})	86 ± 8	79 ± 17	81 ± 22	0.63
LVEF (%)	38 ± 6	37 ± 6	40 ± 11	0.84
Medication				
Angiotensin converting enzyme-inhibitors	5 (50%)	6 (60%)	8 (89%)	0.19
Angiotensin II receptor antagonists	4 (40%)	4 (40%)	1 (11%)	0.30
Aldosterone antagonist	6 (60%)	7 (70%)	8 (89%)	0.36
Diuretics (loopdiuretics)	7 (70%)	6 (60%)	4 (44%)	0.50
β-blockers	10 (100%)	9 (90%)	9 (100%)	0.37
Antiplatelet drugs	6 (60%)	4 (40%)	3 (33%)	0.47
Coumarin derivates	4 (40%)	7 (70%)	4 (44%)	0.35
Statins	10 (100%)	9 (90%)	4 (44%)§	0.007

*Data is presented as mean ± SD. P-values refer to a one-way ANOVA. †Data was unavailable for 1 subject in the CT-group and 3 subjects in the control-group. *Significantly less females compared to the control-group. § Lower compared to CT-group and HIT-group.*

TABLE 2 | Maximal incremental cycling test.

	CT (*n* = 10)		HIT (*n* = 10)		*P*-value		
	Pre	Post	Pre	Post	Time	Type	Time*Type
VO_{2peak} (mL/min)	1881 ± 214	1887 ± 27	1662 ± 562	1792 ± 559	0.06	0.44	0.08
VO_{2peak} (% pred. VO_{2peak})	86 ± 8	87 ± 10	79 ± 17	85 ± 16	0.044	0.48	0.08
Max. workload (Watt)	145 ± 22	152 ± 26	126 ± 38	142 ± 45	< 0.001	0.24	0.07

Data is presented as mean ± SD. P-values refer to a two-way repeated measures ANOVA between the two training groups. One subject in the CT-group did not reach VO_{2peak}, and therefore only VE/VCO_2 slope and VO_2 at AT could be determined.

TABLE 3 | Brachial artery flow-mediated dilation before and after ischaemia-reperfusion injury prior to and after 12-week exercise training.

	Continuous training (*n* = 10)				High-intensity training (*n* = 10)						
	Pre-training		Post-training		Pre-training		Post-training		LMM		
CT (*n* = 10)	Baseline	Post-IR	Baseline	Post-IR	Baseline	Post-IR	Baseline	Post-IR	IR	Training	IR*training
Diameter (mm)	4.5 ± 0.5	4.6 ± 0.5	4.5 ± 0.5	4.5 ± 0.4	4.4 ± 0.9	4.4 ± 0.8	4.4 ± 0.8	4.5 ± 0.6	0.63	0.91	0.99
FMD (%)	5.2 ± 2.5	3.4 ± 1.6*	4.8 ± 3.0	4.2 ± 2.3	5.3 ± 2.6	3.5 ± 1.6*	4.7 ± 2.5	3.8 ± 2.3	0.013	0.90	0.30
FMD_n (%)	5.2 ± 2.4	3.3 ± 1.6*	4.8 ± 2.8	4.2 ± 2.2	5.3 ± 2.4	3.5 ± 1.6*	4.7 ± 2.4	3.8 ± 2.3	0.016	0.91	0.28
SR_{AUC} (A.U., 10^3)	19.9 ± 9.6	20.1 ± 8.2	18.6 ± 7.4	20.6 ± 8.8	17.8 ± 9.2	22.4 ± 9.1	22.3 ± 7.7	17.8 ± 6.5	0.76	0.89	0.33

**Post hoc significantly different from baseline at P < 0.05.*

the ability of exercise training to attenuate endothelial IR-injury is independent on the type of exercise training in HF patients. Our data, therefore, suggest that both types of exercise training improve tolerance of the vasculature against local ischaemia within 12-weeks. Supported by the presence of exaggerated endothelial IR-injury in HF (Ferdinandy et al., 2014; Seeger et al., 2016), but also by the inability of (non)pharmacological interventions to improve these responses (Heusch, 2013), future studies are warranted to further explore the potential meaning and relevance of the ability of exercise training to attenuate endothelial IR-injury in HF patients.

Previous work from both animal and human studies have provided increasing evidence that exercise possesses preconditioning effects (Thijssen et al., 2018). Immediate and

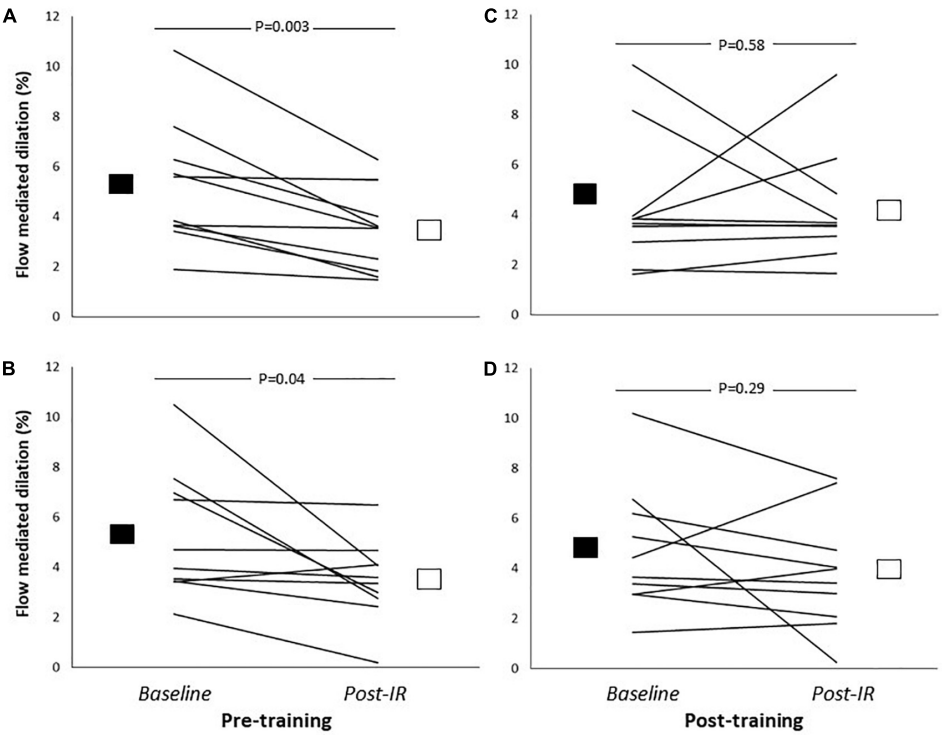

FIGURE 1 | Brachial artery flow-mediated dilation (relative change from resting diameter, %) before (Baseline; solid square) and after 5-min of ischaemic handgrip exercise and 20-min reperfusion (Post-IR; open square) before and after 12-week continuous training (**A,B**: $n = 10$) or high-intensity interval training (**C,D**: $n = 10$). *P*-values refer to *post hoc* analysis related to the impact of IR-injury (LMM effect of IR: $P = 0.01$). Lines represent individual data for all participants.

TABLE 4 | Brachial artery flow-mediated dilation before and after ischaemia-reperfusion injury prior to and after 12-week control period ($n = 9$).

	Before		After		LMM		
	Baseline	**Post-IR**	**Baseline**	**Post-IR**	**IR**	**Time**	**IR*time**
Diameter (mm)	4.1 ± 0.8	4.1 ± 0.8	4.0 ± 0.8	4.1 ± 0.9	0.89	0.93	0.85
FMD (%)	5.3 ± 2.0	3.9 ± 2.2*	5.4 ± 2.4	3.6 ± 1.7*	0.03	0.93	0.77
FMD_n (%)	5.2 ± 1.9	3.9 ± 2.2*	5.4 ± 2.2	3.6 ± 1.7*	0.006	0.80	0.84
SR_{AUC} (A.U., 10^3)	17.7 ± 13.4	19.6 ± 10.8	22.5 ± 15.5	23.5 ± 8.2	0.72	0.29	0.91

**Post hoc significantly different from baseline at $P < 0.05$.*

chronic protective effects of exercise training have been reported, in that a smaller or even abolished decline in endothelial IR-injury is reported in response to acute exercise in healthy young (Seeger et al., 2015) and in physically active older humans (Maessen et al., 2017). To further explore this field, our observation represents the first in the literature in humans that examined and showed that regular exercise training is able, within subjects, to improve tolerance against endothelial IR-injury. Interestingly, these effects were present without changes in baseline FMD, as also described in our previous work (Benda et al., 2015a). The lack of improvement in endothelial function after exercise training in HF patients is not in line with a majority of previous work (Green et al., 2017). Potential explanations for this relate to the relatively low volume and/or frequency of exercise training in our study. Alternatively, explanations relate to the relatively high baseline FMD prior to training [and thus less potential for improvement (Green et al., 2014b)] and/or attenuated shear rate responses during training in HF patients (providing a smaller stimulus for vascular adaptation; Benda et al., 2015b). At least, our observations suggest that benefits of exercise on the vasculature may be mediated through various pathways, including tolerance against potentially harmful stimuli.

The observations from our study raise questions related to the potential mechanisms underlying these observations. Based on the anti-atherogenic characteristics and vasodilator effects of NO, this molecule may contribute to increased tolerance against ischaemia. Indeed, infarct-sparing effects of training were abolished in eNOS-deficient mice (de Waard et al., 2010) and in hearts of trained rats when treated with eNOS inhibitors (Farah et al., 2013). However, we found no changes in brachial artery FMD, a measure that reflects NO-mediated vasodilator function (Green et al., 2014a). An alternative explanation

relates to the ATP-sensitive potassium channels, especially since opening of these channels before IR-injury may protect the heart (Powers et al., 2014). To support this idea, exercise training in animals resulted in a smaller infarct size, whilst sarcoK_{ATP}-blockade, but not mitoK_{ATP}-blockade, abrogated the protective effect (Brown et al., 2005). Since exercise training improves mitochondrial function (Powers et al., 2014) this may contribute to increased tolerance against ischaemia by virtue of the expression of (antioxidant) proteins to minimize ROS formation. Finally, training may affect opioid- and/or adenosine-receptors, especially since the infarct-sparing effects of exercise training can be prevented by blocking (delta) opioid (Michelsen et al., 2012) or adenosine receptors (Domenech et al., 1998).

The second aim of our study was to examine whether the type of exercise affected the effects of exercise training. Based on earlier observations that high-intensity interval exercise has obvious similarities with IPC (i.e., repeated periods of local hypoxia or relative deoxygenation), but also because a single bout of interval but not endurance exercise prevents endothelial IR-injury (Seeger et al., 2015), we expected HIT to lead to superior effects compared to continuous exercise training. Despite these potential differences with acute bouts of exercise, we found that the type of exercise training did not alter our main outcomes. Interestingly, in a previous study it was found that regular resistance training is associated with less endothelial IR-injury in young subjects (DeVan et al., 2011). In another study, lifelong regular endurance exercise training was associated with protection against endothelial IR-injury in an older population (Maessen et al., 2017), a finding also observed by others (Devan et al., 2011). Taken together, our study provides further support for the ability of regular exercise training to attenuate endothelial IR-injury in humans, even in those with cardiovascular disease, whereas the type of exercise training seems less important to achieve these benefits.

Previous work found exaggerated endothelial IR-injury in this population, but also attenuated efficacy of ischaemic preconditioning (Seeger et al., 2016). In line with these findings, no clinical benefit of ischaemic preconditioning interventions have been observed in clinical studies (Ferdinandy et al., 2014). Nonetheless, exercise training was effective in attenuating IR-injury. First, this suggests that the exaggerated decline in endothelial IR-injury is not the result of HF *per se*. Secondly, our observations raise the hypothesis that exercise may represent a more powerful preconditioning stimulus compared to ischaemic preconditioning. Exercise more rapidly induces hypoxia (and across a larger tissue area) compared to repeated cuff inflation around an arm. This could translate to clinically relevant effects, especially since exercise (preconditioning) is easier and more frequently to perform compared to ischaemic preconditioning. Taking these differences into account, exercise may represent a more feasible and effective preconditioning strategy for clinical use than traditional ischaemic preconditioning using blood pressure cuffs.

Limitations

An obvious limitation of our study it the inability to translate our model of endothelial IR-injury to cardiac tissue and/or (non)fatal tissue damage. Nonetheless, this model is frequently used, whilst results related to preconditioning stimuli match observations from both pre-clinical and clinical observations. Nonetheless, future work is required to better understand these effects and improve translation (and mechanistic insight) of our results. Another limitation of our study is the relatively small number of patients. Nonetheless, our sample size was not different from other studies examining the impact of (various types of) exercise training (Wisloff et al., 2007; Fu et al., 2013; Ramos et al., 2015). Importantly, we have adopted state-of-the-art techniques for and followed expert-consensus guidelines to assess vascular function. Although our sample size may have been insufficient to detect differences between the two types of training, previous work also suggested that moderate- and high-intensity training lead to comparable recovery of cardiac function after cardiac ischaemia (Lennon et al., 2004).

CONCLUSION

Our results reveal that 12-weeks exercise training in HF patients leads to attenuation in endothelial IR injury, an effect that is independent of the type of exercise training (continuous endurance vs. high-intensity interval). Although exercise training did not alter resting endothelial function, our data indicate that regular exercise training improves tolerance against endothelial IR injury. These changes may contribute to the cardioprotective effects of exercise training. Moreover, in light of the disappointing results from clinical studies adopting ischaemic preconditioning, exercise preconditioning may be a more potent, but also easier and freely applicable, stimulus for cardioprotection in humans.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

NB, JS, AvD, MH, and DT conceived and designed the research. NB, JS, and AvD contributed to recruitment of participants. NB, JS, AvD, and DT acquired and analyzed the data. NB, AvD, MH, and DT interpreted results of the research. All authors edited and revised the manuscript, and approved final version of the manuscript.

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Tissue Oxygenation in Response to Different Relative Levels of Blood-Flow Restricted Exercise

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Blood flow restrictive (BFR) exercise elicits a localized hypoxic environment compatible with greater metabolic stress. We intended to compare the acute changes in muscle microvascular oxygenation following low-intensity knee extension exercise, combined with different levels of BFR. Thirteen active young men (age: 23.8 ± 5.4 years) were tested for unilateral knee extension exercise (30 + 15 + 15 + 15 reps at 20% one repetition maximum) on four different conditions: no-BFR (NOBFR), 40, 60, and 80% of arterial occlusion pressure (AOP). Deoxyhemoglobin+myoglobin concentration Deoxy[Hb+Mb], total hemoglobin [T(H+Mb)] and tissue oxygen saturation [TOI] were measured on the vastus lateralis muscle using near-infrared spectroscopy (NIMO, Nirox srl, Brescia, Italy). The magnitude of change in Deoxy[Hb+Mb]during exercise was similar between 60 and 80% AOP. Overall, compared to that seen during 60 and 80% AOP, NOBFR as well as 40% AOP resulted in a lower magnitude of change in Deoxy[Hb+Mb] ($p < 0.05$). While the oxygen extraction decreased during each inter-set resting interval in NOBFR and 40% AOP, this was not the case for 60 or 80% AOP. Additionally, TOI values obtained during recovery from each set of exercise were similarly affected by all conditions. Finally, our data also show that, when performed at higher restrictive values (60 and 80%), BFR exercise increases total Deoxy[Hb+Mb] extraction ($p < 0.05$). Taken together, we provide evidence that BFR is effective for increasing deoxygenation and reducing tissue oxygenation during low-intensity exercise. We also showed that when using low loads, a relative pressure above 40% of the AOP at rest is required to elicit changes in microvascular oxygenation compared with the same exercise with unrestricted conditions.

Keywords: muscle oxygenation, KAATSU, resistance exercise, near-infrared spectroscopy, oxygen extraction

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INTRODUCTION

The chronic effect of using tourniquet cuffs to restrict muscle blood flow during resistance exercise on muscle size and strength are well established in the available literature (Loenneke et al., 2012; Slysz et al., 2016). Despite significant efforts to fully comprehend the physiological basis of muscle growth resulting from blood flow restricted (BFR) exercise, the specific mechanisms underlying such response remain largely unknown (Manini and Clark, 2009). There is general

agreement that muscle hypertrophy is triggered by the combined effect of mechanical tension, muscle damage and metabolic stress (Schoenfeld, 2010). Several studies have shown that performing BFR exercise with 20% of 1RM induces the same or even greater fatiguing stimulus when compared with high intensity exercise training (Takarada et al., 2000; Cook et al., 2007). Thus, it can be used in multiple exercise settings, particularly when high intensity resistance exercise is not recommended (Cook et al., 2007). Since BFR exercise is typically performed using light loads, metabolic stress is believed to be largely responsible for enhancements in both muscle size and strength after training (Takarada et al., 2000, 2002).

Past research has shown that microvascular oxygenation varies as a function of BFR absolute pressure and duration (Karabulut et al., 2014; Neto et al., 2014). Nevertheless, it is important to note that BFR exercise is typically structured using a predetermined number of sets and repetitions and that, within this context, the magnitude of neuromuscular activation and fatigue varies as a function of BFR relative pressure (Fatela et al., 2016). For this reason, several reports focused on exploring the overall impact (i.e., during and between sets) of BFR on microvascular oxygenation during low-intensity resistance exercise (Ganesan et al., 2015; Lauver et al., 2017; Yanagisawa and Sanomura, 2017). Their findings are somewhat conflicting, and this is probably a consequence of differences between methodological designs (e.g., exercise selection, BFR level as well as duration, contraction mode and site for monitoring tissue oxygenation). To our knowledge, only one previous investigation examined the role of relative BFR pressure on microvascular oxygenation (Kilgas et al., 2018). Using handgrip exercise, it was shown that setting BFR to 60 and 80% of the pressure required to block arterial blood flow, or arterial occlusion pressure (AOP) elicited a reduction in tissue saturation index and an increase in deoxyhemoglobin+myoglobin concentration (Deoxy[Hb+Mb]). However, due to the characteristics of this specific exercise paradigm, these findings may not be extensive to dynamic exercise involving larger muscle mass. Determining the optimal level of BFR pressure, relative to AOP, is fundamental for maximizing the effectiveness of exercise prescription because it might influence the possible mechanism for the adaptations following BFR training (Cayot et al., 2014; Scott et al., 2015).

Therefore, we aimed at comparing the acute response of Deoxy[Hb+Mb], total hemoglobin+myoglobin [T(H+Mb)] and tissue oxygen saturation (TOI) in the vastus lateralis muscle during low-intensity exercise performed at different levels of relative BFR. We hypothesized that, during dynamic knee extension exercise, the peripheral deoxygenation would increase, and total hemoglobin+myoglobin would increase as a function of the percent arterial occlusion pressure.

MATERIALS AND METHODS

Participants

Thirteen active young men (age: 23.8 ± 5.4 years; height: 174.8 ± 4.2 cm; body mass: 69.8 ± 7.0 kg; Systolic blood pressure: 122.6 ± 7.0 mmHg; diastolic blood pressure: 76.7 ± 8.5 mmHg; AOP: 136.6 ± 9.3 mmHg) volunteered to participate in this study. The participants were fully informed of any risk and discomfort associated with the experiments before providing written consent. Participants were not enrolled in any kind of resistance or endurance training in the 6 months prior to the participation in the study. They were all non-smokers and free from any known cardiovascular and metabolic diseases, as assessed by medical history. The participants were instructed to maintain the same level of physical activity throughout the course of the study. They were asked to avoid exercise as well as the intake of caffeine and alcohol for at least 24 h before testing. Testing was performed at the same time of day for standardization purposes with at least 48 h between testing sessions. This study was approved by the Faculty's Ethics Committee (CEFMH 17/2014) and in accordance with the Declaration of Helsinki.

Experimental Design

All participants were familiarized with the testing procedures in two separate sessions. Metronome pacing and AOP measurements were performed during the first session. Then, on a different day, all participants performed a baseline session, where (a) AOP for the right lower limb was reassessed, (b) one repetition maximum (1RM) was determined for the right knee extensors and (c) testing (including metronomic pacing) was again reproduced. Using a crossover design, participants then visited the laboratory on four additional days to complete the following resistance exercise trials in a randomized order: without BFR (NOBFR), 40, 60, and 80% AOP. Participants were tested in the seated position and were fixed with chest and abdominal straps. Vascular restriction was elicited using a 13 × 124 cm pneumatic cuff (SC12L Tourniquet Cuffs, D. E. Hokanson, Inc., Bellevue, WA), applied to the most proximal portion of the right thigh and the pressure was maintained throughout the test.

The exercise protocol was performed using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY), and consisted of a series of unilateral knee extensions at 20% of 1RM throughout all testing sessions. This training intensity was selected because of its effectiveness in inducing fatigue when combined with BFR (Takarada et al., 2000; Cook et al., 2007). During exercise, participants were monitored for microvascular oxygenation using near-infrared spectroscopy (NIRS) (NIMO, Nirox srl, Brescia, Italy). Knee-extension peak torque was also determined during maximal voluntary isometric contraction (MVIC) before and after acute exercise.

Determination of AOP

Arterial occlusion pressure was determined using a vascular Doppler probe (PD1+ Combi, Ultrasound Technologies Ltd., Caldicot, United Kingdom) placed over the right posterior tibial artery, halfway between the posterior border of the medial malleolus and the Achilles tendon. A pneumatic cuff was inflated (E20/AG101 Rapid Cuff Inflator) gradually up to the point when the auscultatory pulse of the posterior tibial artery was interrupted (Fatela et al., 2018). To guarantee similar

cuff placement between familiarization and testing sessions, a photographic record was made for each participant.

1RM Testing

The maximal torque produced in a single repetition was determined for the right knee extension using the isotonic mode of the isokinetic dynamometer. The participants were asked to complete one repetition through a full range of motion (90°). Strong verbal encouragement was given in each trial, and 2 min of recovery were allowed between attempts. 1RM was always determined within five trials (mean value: 219.2 ± 37.8 Nm).

Maximal Voluntary Isometric Contraction

Maximal voluntary isometric contraction was determined at the optimal joint angle for right knee extension in all testing sessions. Participants performed three isometric knee extension trials (3 s per trial). They were instructed to exert their maximum force as fast and hard as possible. One min of recovery between trials was allowed. Peak torque was set as pre- and post-exercise MVIC (2 min post-cuff deflation).

Exercise Protocol

After a standardized warm-up (6 min of unloaded cycle-ergometry), participants performed 4 sets of knee extension at 20% 1RM (30 + 15 + 15 + 15 reps, set 1–4, respectively), with 30 s of passive rest between sets (rest 1–3) (Yasuda et al., 2008; Loenneke et al., 2013). A metronome was used to control the concentric–concentric mode, with 1 s for knee extension (20% 1-RM) and another for knee flexion (unloaded). Verbal encouragement was provided to warrant that each participant completed the full exercise protocol. For safety reasons, a pulse oximeter (Onyx® II 9560, Nonin Medical Inc., Plymouth, MN) was placed in the right *hallux*, immediately after each set to ensure that blood flow was not completely halted by tissue edema. In the BFR sessions, the cuff was inflated before exercise.

NIRS Signal

Near-infrared spectroscopy provides non-invasive information about the changes in oxygenation and hemodynamics in muscle tissue based on the oxygen-dependent characteristics of near-infrared light (Perrey and Ferrari, 2018) and has been validated for multiple forms of resistance exercise (Pereira et al., 2007). NIRS measurements were performed on the vastus lateralis muscle of the right leg throughout the entire duration of the exercise protocol. The skin of each participant's right leg was initially cleaned and shaved. Then, the probe was placed on the belly of the muscle, midway between the lateral epicondyle and greater trochanter of the femur. The placement of the probe was marked with indelible ink and a photographic recording was taken to ensure similar placement of the probe between sessions. The probe was attached to the skin surface with tape and then covered with an optically dense elastic bandage. This minimized its movement and prevented the intrusion of extraneous light and loss of near NIR-transmitted light from the field of interrogation.

Deoxy[Hb+Mb], oxy hemoglobin+myoglobin [(Hb+Mb)O_2] and [T(Hb+Mb)] were quantified with a continuous-wave tissue oximeter (NIMO, Nirox srl, Brescia, Italy). TOI was expressed in % and calculated as:

$$[(Hb + Mb)O2]/[(Hb + Mb)O2] + Deoxy[Hb + Mb] \times 100. \quad (1)$$

Briefly, this system is based on the O_2 dependency of absorption changes for near infra-red light in hemoglobin and myoglobin and it consists on an emission probe which emits three wave lengths (685, 850, and 980 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 40 Hz and used to estimate Deoxy[Hb+Mb], (1) [(Hb+Mb)O2] and [T(Hb+Mb)](Rovati et al., 2004; Ferrari et al., 2011). As the NIRS signal is unable to distinguish between hemoglobin and myoglobin, results are considered as the combined O_2 saturation of these metalloproteins. To account for the possible influence of the local fat layer on NIRS, a real-time correction using an algorithm included in the software (Nimo Data Analysis Peak) was used. Deoxy[Hb+Mb]signal is less dependent of changes in blood flow and is considered as an indicator of fractional O_2 extraction within the microvascular level (Ferrari et al., 1997). [T(Hb+Mb)] reflects the total amount of hemoglobin, and it can be interpreted as changes in blood volume within the tissue vascular beds (Van Beekvelt et al., 2001). TOI reflects the dynamic balance between O_2 supply and O_2 uptake and is independent of near-infrared photon path length in muscle tissue (Brocherie et al., 2015).

Data Handling

Baseline values for each testing session were established as 1-min averages for NIRS derived signal. To minimize the impact of baseline differences between trials, Deoxy[Hb+Mb] and [T(Hb+Mb)] values were computed as changes (Δ) from baseline within each testing session. Deoxy[Hb+Mb] and [T(Hb+Mb)] and TOI were measured throughout the exercise protocol. For these analyses, sets were divided into thirds and mean value ± SD of the last third was used as representative of each correspondent phase. (Set 1, Recovery 1, Set 2, Recovery 2, Set 3, Recovery 3, Set 4). Δ [T(Hb+Mb)] was also analyzed for the recovery periods.

Relative recovery (%Rec) for Deoxy[Hb+Mb] and [T(Hb+Mb)] and TOI was determined as the % difference between the last third of recovery and the last third of the previous set. Total oxygen extraction was computed as the area under the Deoxy[Hb+Mb] curve including all sets and recovery periods (Soares et al., 2017).

Statistical Analysis

Data are reported as mean ± SD, unless otherwise specified. The Mauchly's test was used to test the assumption of sphericity. The Greenhouse-Geisser correction was implemented to adjust the degrees of freedom for the averaged tests of significance when the assumption of sphericity was not met. Paired *t*-tests were used for exploring possible differences in peak torque from pre- to post-exercise at each condition (NOBFR, 40, 60, and 80% AOP). A two-way repeated measures ANOVA [four conditions (NOBFR vs. 40 vs. 60 vs. 80% AOP) × 5 times (Pre-exercise vs. set 1 vs. set 2 vs. set 3 vs. set 4)] was used

to determine the impact of BFR on each dependent variable (i.e., Deoxy[Hb+Mb] and [T(Hb+Mb)] and TOI). Another two-way repeated measures ANOVA [four conditions (NOBFR vs. 40 vs. 60 vs. 80% AOP) × 3 times (recovery 1 vs. recovery 2 vs. recovery 3)] was additionally used to determine the impact of each condition on the %Rec of Deoxy[Hb+Mb] and [T(Hb+Mb)] and TOI. When a significant effect was detected at a significance level of $p < 0.05$, *t*-tests were used for *post hoc* comparisons. Adjustment for multiple comparisons was made with Bonferroni's correction. All statistical calculations were computed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL). Significance was set at $p < 0.05$.

RESULTS

Peak torque was similar between pre- and post-exercise time points in all conditions, except for 80% AOP (reduction of 5.8%, $p < 0.05$). **Tables 1–3** show the changes of Deoxy[Hb+Mb] and [T(Hb+Mb)] and TOI in transition from baseline to exercise (sets 1–4). We obtained a condition-by-time interaction for Deoxy[Hb+Mb] and [T(Hb+Mb)] ($F = 12.4$ and 10.3, $p < 0.05$; respectively). *Post hoc* analyses indicate that [HHb] increased from pre-exercise to set 1 in both NOBFR and 40% AOP ($p < 0.05$). No other changes were noted for this parameter during exercise. With the exception of pre-exercise, both these conditions exhibited similar Deoxy[Hb+Mb] values over time. As importantly, Deoxy[Hb+Mb] remained unchanged over time during exercise performed at 60 and 80% AOP. Nevertheless, Deoxy[Hb+Mb] values were consistently lower during NOBFR and 40% AOP when compared to that seen at 60 and 80% AOP throughout all time points ($p < 0.05$). Finally, the acute response of Deoxy[Hb+Mb] to low-intensity exercise was similar between 60 and 80% AOP.

[T(Hb+Mb)] decreased with BFR exercise ($p < 0.05$) and then remained stable after set 1. Conversely, no changes were seen in [T(Hb+Mb)] in response to NOBFR exercise. There were significant differences in pre-exercise [T(Hb+Mb)] between NOBFR, AOP 40 and 60% ($p < 0.05$). Conversely, this was not the case for comparisons between AOP 60 and 80%. Even though, all conditions attained similar endpoints at the completion of set 1 and 4, [T(Hb+Mb)] was higher in 60 vs. 40% AOP after set 2 and 3 ($p < 0.05$). In Set 3, [T(Hb+Mb)] was also increased following exercise with 60% AOP vs. NOBFR ($p < 0.05$).

There were significant main effects of condition and time for TOI ($F = 4.3$ and 63.0, respectively; $p < 0.05$). Follow up analyses revealed that TOI was lower in 80% AOP than in NOBFR ($p < 0.05$). No other differences were seen between conditions. Additionally, TOI decreased in all conditions from pre-exercise to set 1 ($p < 0.05$) and then remained stable until the end of set 4.

TABLE 1 | Delta changes in deoxygenated hemoglobin+myoglobin (AU) (Mean and ± standard deviation) for before exercise (Pre) and Set 1–4 in each blood flow restrictive pressure.

	Pre	Set 1	Set 2	Set 3	Set 4
NOBFR	−2.3 ± 4.9	19.4 ± 8.8[a]	18.9 ± 11.0	19.3 ± 11.3	19.5 ± 11.2
40% AOP	11.0 ± 4.8*	21.2 ± 8.8[a]	20.0 ± 8.3	19.9 ± 8.6	20.2 ± 8.4
60% AOP	18.7 ± 10.9*§	27.3 ± 10.9*§	27.6 ± 11.5*§	27.8 ± 12.0*§	27.3 ± 11.8*§
80% AOP	25.6 ± 10.9*§	27.3 ± 12.1*§	27.7 ± 12.3*§	27.2 ± 12.6	27.5 ± 12.4*§

**Significantly different from NOBFR; § Significantly different from 40% AOP; [a]Significantly different from PRE; (p < 0.05).*

TABLE 2 | Delta changes in total hemoglobin+myoglobin (AU) (Mean and ± standard deviation) for before exercise (Pre) and Set 1–4 in each blood flow restrictive pressure.

	Pre	Set 1	Set 2	Set 3	Set 4
NOBFR	−2.5 ± 13.2	−5.0 ± 14.9	−3.7 ± 15.3	−6.3 ± 16.6	−3.8 ± 18.1
40% AOP	24.1 ± 15.0*	−4.6 ± 16.6[a]	−4.3 ± 14.2	−4.4 ± 18.6	−0.8 ± 17.8
60% AOP	45.1 ± 24.6*§	6.8 ± 14.1[a]	12.4 ± 14.0§	13.2 ± 14.6*	13.5 ± 18.0
80% AOP	48.5 ± 23.0*§	−0.8 ± 18.7[a]	8.5 ± 14.5	6.6 ± 15.8	12.3 ± 13.4

**Significantly different from NOBFR; § Significantly different from 40% AOP; [a]significantly different from PRE; (p < 0.05).*

TABLE 3 | Tissue oxygenation index (AU) (Mean and ± standard deviation) before exercise (Pre) and Set 1–4 in each blood flow restrictive pressure.

	Pre	Set 1	Set 2	Set 3	Set 4
NOBFR	80.6 ± 3.5	65.2 ± 9.5[a]	65.9 ± 11.1	65.0 ± 11.1	65.6 ± 10.2
40% AOP	72.7 ± 7.4	58.1 ± 13.9[a]	59.0 ± 14.4	59.1 ± 14.4	60.4 ± 12.7
60% AOP	73.1 ± 7.5	58.9 ± 14.2[a]	60.5 ± 13.6	61.0 ± 12.6	61.5 ± 12.8
80% AOP	69.7 ± 8.9	55.6 ± 13.8[a]	58.5 ± 13.1	58.3 ± 13.3	59.9 ± 11.9*

**Significantly different from NOBFR; [a]significantly different from PRE; (p < 0.05).*

During the inter-set recovery intervals, the relative changes from baseline in [T(Hb+Mb)] presented a significant main effect for condition ($F = 21.7$; $p < 0.01$). Follow up analyses revealed that this variable increased progressively with the restrictive pressures. However, there were no significant differences between 60 and 80% AOP (**Table 4**).

As depicted in **Figure 1**, depending on the condition, there were different levels of muscle oxygenation during each inter-set recovery period. Specifically, while Deoxy[Hb+Mb] exhibited a high level of recovery following each NOBFR and 40% AOP exercise set, this was not the case for 60 nor 80% AOP ($p < 0.05$). There was virtually no recovery in Deoxy[Hb+Mb] after 60% AOP and, in 80% AOP, the level of oxygen extraction actually increased. TOI exhibited a significant recovery in all conditions during the inter-set periods ($p < 0.05$). The magnitude of TOI recovery was similar between NOBFR and 40% AOP and between 60 and 80% AOP, respectively (**Figure 2**). [T(Hb+Mb)] showed no significant differences between conditions for the percentage of recovery from each set.

The area under the Deoxy[Hb+Mb]curve was used to explore the impact of each exercise protocol on total oxygen extraction. As can be seen in **Figure 3**, the area under the Deoxy[Hb+Mb] curve increased as a function of the percentual AOP pressure used ($p < 0.05$). However, it should be noted that the differences in this parameter did not achieve significance for comparisons between 60 and 80% AOP ($p > 0.05$).

TABLE 4 | Delta changes in total hemoglobin+myoglobin (AU) (Mean and ± standard deviation) for Recovery 1–3 in each blood flow restrictive pressure.

	Recovery 1	Recovery 2	Recovery 3
NOBFR	6.6 ± 10.3	4.3 ± 13.8	4.1 ± 16.2
40% AOP	26.8 ± 13.2	25.0 ± 17.8	25.1 ± 20.5*
60% AOP	43.8 ± 13.0	41.7 ± 12.0	43.4 ± 15.7*§
80% AOP	41.5 ± 17.6	40.9 ± 15.3	45.2 ± 16.9*§

**Significantly different from NOBFR; § Significantly different from 40% AOP ($p < 0.05$).*

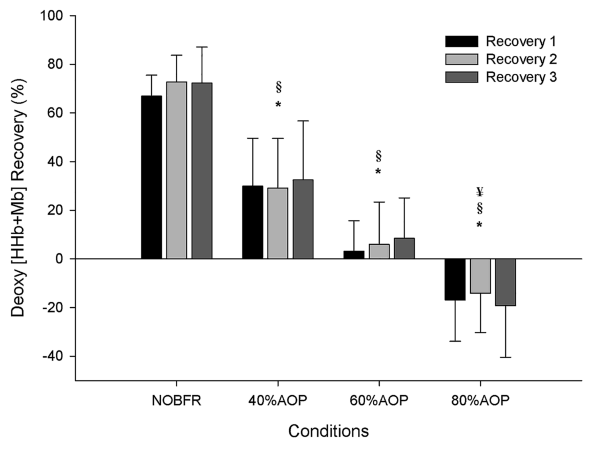

FIGURE 1 | Deoxygenation percentage of recovery during inter-set intervals. *Significantly different from NOBFR; § Significantly different from 40% AOP; ¥ Significantly different from 60% AOP; $p < 0.05$.

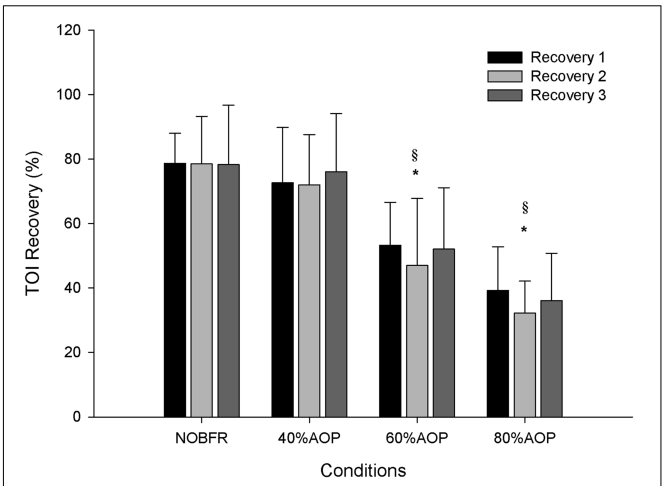

FIGURE 2 | Tissue oxygenation percentage of recovery during inter-set intervals. *Significantly different from NOBFR; § Significantly different from 40% AOP; $p > 0.05$.

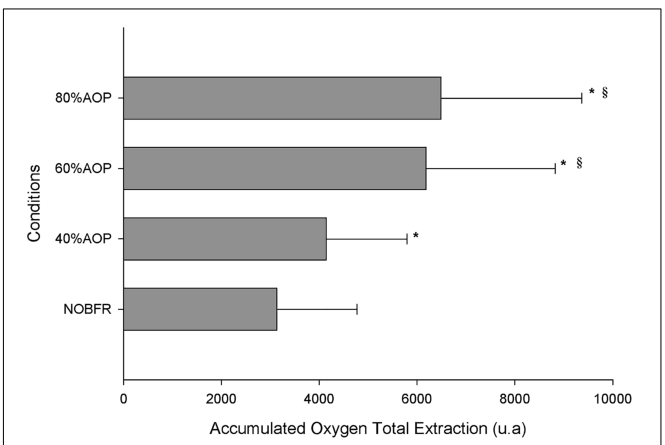

FIGURE 3 | Total oxygen extraction represented as accumulated change from baseline in Deoxy[Hb+Mb] *Significantly different from NOBFRE; § Significantly different from 40% AOP; $p > 0.05$.

DISCUSSION

We explored the interaction between different levels of BFR, relative to AOP, and muscle oxygenation in response to an acute multi-set knee-extension exercise protocol. It was demonstrated that BFR increases deoxygenation and hampers tissue oxygenation during low-intensity muscle contractions. Thus, our hypotheses were partially confirmed. However, we also showed that to induce a considerable stress on microvascular oxygenation, represented by increased deoxygenation levels, using low loads (20% RM), BFR should be set above 40% of AOP. Otherwise, the level of muscle oxygenation/deoxygenation is not substantially different from that seen during non-BFR exercise. Since we did not test pressures between 40 and 60% AOP, we can only say that in the three pressures studied, 60% AOP appears to represent a threshold required to induce higher

deoxygenation and decreased tissue oxygenation levels within this form of resistance training. As importantly, we provide evidence that setting BFR to 80% of AOP exerts no further impact in altering microvascular deoxygenation compared to that seen using 60%. This is important because it corroborates past findings on BFR-induced muscle fatigue and activation (Fatela et al., 2016).

The use of different restrictive pressures interacts with muscle activation and neuromuscular fatigue (Loenneke et al., 2015; Fatela et al., 2016). According to our findings, this is also the case for microvascular oxygenation during acute low-intensity BFR exercise. BFR is thought to reduce venous return, while eliciting a turbulent flow in the arterial circulation. Ultimately, this decreases blood flow velocity within the tissues distal to the cuff (Manini and Clark, 2009). There is general agreement that the acute impact of BFR induces local hypoxia as well as an accumulation of metabolites (resulting from increased production and limited removal) (Scott et al., 2015). Our findings of increased deoxygenation and lower tissue O_2 saturation, obtained during exercise with 60 and 80% AOP, further support this concept. This is the first study focusing on the interaction between BFR and tissue oxygenation, using a typical and well-validated exercise protocol (Loenneke et al., 2014a; Scott et al., 2015). Past reports have used NIRS measurements to examine muscle oxygenation during handgrip, eccentric, isometric or limited volume exercise as well as in response to muscle contractions performed to failure (Cayot et al., 2014; Ganesan et al., 2015; Lauver et al., 2017; Yanagisawa and Sanomura, 2017; Kilgas et al., 2018). Accordingly, comparisons between our results and those of the available research are challenging. Nevertheless, using an exercise protocol combining CON and ECC muscle contractions, Lauver et al. (2017), also showed heightened deoxygenation with BFR set at 130% of resting systolic blood pressure. Similar findings were also reported for low-volume isometric exercise, combined with BFR set at 60 and 80% of AOP (Cayot et al., 2014). Conversely, Ganesan et al. (2015) did not find a higher muscle oxygen extraction in BFRE knee extension exercise sets when compared with time matched unrestricted exercise. However, these authors prescribed an absolute and arbitrary BFR value of 100 mm Hg, which most likely affected their findings.

In our study, the differences observed in the Deoxy[Hb+Mb] response were noticeable before the beginning of exercise – a time point representing the effect of BFR on microvascular oxygenation *per se*. Using 60 and 80% AOP resulted in higher values of Deoxy[Hb+Mb] throughout the testing protocol. While Kilgas et al. (2018) showed a progressive increase in Deoxy[Hb+Mb] during exercise set at 60 and 80% AOP, we found that, under these specific circumstances, deoxy[Hb+Mb] remained unaltered from pre-exercise to the end of each set. Such discrepancies might be secondary to the differences between studies in the total length of time exposure to BFR (longer in our study). As importantly, Kilgas et al. (2018) analyzed only 1 set of isolated handgrip exercise for each restrictive pressure and all participants exercised using several levels of BFR within the same day. Thus, data from this study are most likely contaminated by the BFR-associated reactive hyperaemia (Mouser et al., 2017). However, we must acknowledge that we did not establish the level of venous return restriction induced by the different restrictive pressures. Therefore, we cannot establish on what extent the increased extraction in 60 and 80% AOP was due to the limited venous return.

We calculated the accumulated Deoxy[Hb+Mb] variation across the total exercise protocol. This represents the total oxygen extraction occurring during both exercise and rest periods (Soares et al., 2017). We found that the accumulated Deoxy[Hb+Mb] increased in parallel with BFR relative pressure. However, there were no differences between the impact of 60 and 80% AOP. Although we did not measure the pressure required to occlude venous return, we speculate that above 60% AOP there was no added effect; thus explaining the *plateau* on deoxy [HHb+Mb]. In fact, previous research shows that venous occlusion occurs at somewhat lower pressures. For example, it may be induced at an absolute pressure of 60 mmHg or even at 20 mmHg below diastolic blood pressure. Moreover, it has also been shown that BFR pressures between 45 and 200 mmHg produce a similar effect on reducing left ventricular end-diastolic volume – thus corroborating the concept that venous occlusion is attained at low levels of cuff pressure (Iida et al., 2005; Corrigan et al., 2008). In support of this, in most studies focusing on measuring blood flow to the lower limb using plethysmography, 50 mmHg is generally used as effective for inducing total venous occlusion (Patterson and Ferguson, 2010), and this is considerably lower than the pressure used at 60% AOP. In fact, the pressure that we used to induce 60% AOP was > than 50 mmHg and > than diastolic blood pressure. Conceptually, this confirms that there is no added restriction of venous return between 60 and 80% AOP. Additionally, we found that in the recovery periods between sets, the two higher pressures presented significantly higher values of T[HHb+Mb], which can be considered a proxy for blood flow. However, there were no differences between 60 and 80% AOP in this variable.

It has been hypothesized that the optimal BFR pressure may follow a hormetic-like relationship (Loenneke et al., 2014b; Scott et al., 2015; Kilgas et al., 2018), with some literature postulating that high pressures may not enhance muscular development more than moderate pressures. Even though recent data suggest that BFR exercise protocols may benefit from higher levels of restriction (80%) when exercising at very low intensities (Lixandrão et al., 2015), the authors only compared the effects of 12 weeks of training with either 40 or 80% AOP. Our data are in accordance with those of Kilgas et al. (2018) for arm exercise in that 60% AOP might represent a physiological threshold for tissue hypoxia and metabolite accumulation during low-intensity exercise.

[T(Hb+Mb)], which is considered as a surrogate index of changes in tissue blood volume (Grassi et al., 1999; Van Beekvelt et al., 2001; Perrey and Ferrari, 2018), was also influenced by different levels of restrictive pressures before exercise. Specifically, heightened restrictive pressures induced

progressive increases in [T(Hb+Mb)], which can be attributed to the venous pooling distal to the cuff (Pope et al., 2013; Lauver et al., 2017). However, at a BFR level > than 60% of AOP we observed no further increase in [T(Hb+Mb)], which could result from similar degrees of venous return constrains in these two restrictive pressures. However, it is important to note that 80% AOP was the only condition compatible with a lower TOI and a small, but significant decrease in peak torque post-exercise. Since Deoxy[Hb+Mb] and [T(Hb+Mb)] were similar between 60 and 80% AOP, the greater magnitude of reduction in TOI values at higher levels of relative restriction was most likely caused by further reductions in arterial inflow.

In contrast to that seen during exercise, the inter-set recovery periods were characterized by considerable differences between conditions even at higher percentual of AOP. Globally, our data indicate that recovery of muscle deoxygenation resulting from each set of contractions varies depending on the magnitude of restrictive pressure. Specifically, while the relative recovery of Deoxy[Hb+Mb] was similar between NOBFR and 40% AOP, considerable differences were obtained when comparing 60 to 80% AOP. Specifically, we found that after low-intensity exercise performed at 60% AOP, Deoxy[Hb+Mb] does not recovery after 30 s of pause between sets. Conversely, when exercising at 80% AOP, muscle deoxygenation was actually potentiated during each inter-set rest interval. Although beyond the scope of this study, we speculate that this might be secondary to enhancements in the O_2 deficit resulting from exercise performed at higher levels of BFR (Mendonca et al., 2015; Conceição et al., 2018).

According to the existent literature, the metabolic stress induced from multiple sets of BFR exercise is similar to that seen during high-intensity exercise, but only if the cuff inflation is maintained during recovery from each set (Suga et al., 2012). This approach ensures heightened muscle activation and maximizes training adaptations, independently of the external load (Loenneke et al., 2011). Although we did not compare this setting with a protocol where the cuff was release between sets, our results seems to be in line with the literature. However, we showed that all conditions were compatible with some recovery in tissue oxygenation during each inter-set period. Yet, there was a more pronounced recovery of TOI in NOBFR and 40% AOP compared to that seen during 60 and 80% AOP. Thus, we contend that, for ensuring metabolite buildup during exercise, the cuff should be kept inflated at a level ≥60% AOP.

Limitations

There are several limitations in this study. For instance, the contribution of myoglobin desaturation is not possible to determine via NIRS. Thus, the differences between blood-muscle oxygen transport are not distinguishable in the current study (Ferrari et al., 2011). Additionally, NIRS signal is influenced by the thickness of adipose tissue and by the effect of changes in blood volume along within the tissue (Ferrari et al., 2004). Furthermore, even though we took precautionary measures to improve the reproducibility of probe placement (i.e., using indelible ink and taking photographic records), the day-to-day variation of these specific parameters corresponds to ∼ 8.0–9.4% (Willis et al., 2017). Nevertheless, we are confident that our data were not affected by this, because the use of relative changes in the NIRS variables, which is common in the research area, allows valid comparisons between settings (Perrey and Ferrari, 2018).

We did not establish the venous return occlusion pressure nor the deoxygenation response in full arterial occlusion, which could have provided a better understanding of the similarities between 60 and 80% AOP.

CONCLUSION

Our findings indicate that a relative pressure above 40% of the AOP at rest seems to be required to potentiate the metabolic stress of low-intensity knee extension exercise. Second, they also provide evidence that with the cuff inflated between sets, there is a hampered recovery of tissue oxygenation and deoxygenation levels between higher and lower BFR pressures. Third, they also demonstrate that BFR ≥ 60% of AOP does not enhance oxygen extraction during low-intensity exercise. Fourth, we found an interaction between the magnitude of BFR (from 60 to 80%) and the recovery of muscle deoxygenation during the inter-set rest intervals. Thus, while deoxygenated [HHb+Mb] remains virtually unchanged during recovery from each set of exercise performed at 60% AOP, its values actually increase when using 80% AOP.

ETHICS STATEMENT

This study was approved by the Faculty's Ethics Committee (CEFMH 17/2014) and in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

JR, PF, GM, MV, PM-H, and FA conceived and designed the experiments. JR, PF, MV, and JI performed the experiments. JR, PF, JV, JI, and FA analyzed the data. JR, PF, JV, PM-H, and FA interpreted results of research. JR, JV, MV, and PF drafted the manuscript and prepared the tables and figures. JR, PF, GM, JV, MV, JI, PM-H, and FA edited, critically revised the manuscript and approved the final version of manuscript.

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The Association Between Muscle Deoxygenation and Muscle Hypertrophy to Blood Flow Restricted Training Performed at High and Low Loads

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The metabolic stress induced by blood flow restriction (BFR) during resistance training (RT) might maximize muscle growth. However, it is currently unknown whether metabolic stress are associated with muscle hypertrophy after RT protocols with high- or low load. Therefore, the aim of the study was to compare the effect of high load RT (HL-RT), high load BFR (HL-BFR), and low load BFR (LL-BFR) on deoxyhemoglobin concentration [HHb] (proxy marker of metabolic stress), muscle cross-sectional area (CSA), activation, strength, architecture and edema before (T1), after 5 (T2), and 10 weeks (T3) of training with these protocols. Additionally, we analyzed the occurrence of association between muscle deoxygenation and muscle hypertrophy. Thirty young men were selected and each of participants' legs was allocated to one of the three experimental protocols in a randomized and balanced way according to quartiles of the baseline CSA and leg extension 1-RM values of the dominant leg. The dynamic maximum strength was measured by 1-RM test and vastus lateralis (VL) muscle cross-sectional area CSA echo intensity (CSA_{echo}) and pennation angle (PA) were performed through ultrasound images. The measurement of muscle activation by surface electromyography (EMG) and [HHb] through near-infrared spectroscopy (NIRS) of VL were performed during the training session with relative load obtained after the 1-RM, before (T1), after 5 (T2), and 10 weeks (T3) training. The training total volume (TTV) was greater for HL-RT and HL-BFR compared to LL-BFR. There was no difference in 1-RM, CSA, CSA_{echo}, CSA_{echo}/CSA, and PA increases between protocols. Regarding the magnitude of the EMG, the HL-RT and HL-BFR groups showed higher values than and LL-BFR. On the other hand, [HHb] was higher for HL-BFR and LL-BFR. In conclusion, our results suggest that the addition of BFR to exercise contributes to neuromuscular adaptations only when RT is performed with low-load. Furthermore, we found a significant association between the changes in [HHb] (i.e., metabolic stress) and increases in muscle CSA from T2 to T3 only for the LL-BFR, when muscle edema was attenuated.

Keywords: electromyography, muscle oxygenation, vascular occlusion, muscle hypertrophy, muscle strength

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INTRODUCTION

Resistance training- (RT) induced changes in muscle strength are partially due to increases in muscle cross-sectional area (CSA) (i.e., muscle hypertrophy) and changes in muscle architecture (e.g., increase in the pennation angle of muscle fibers) (Aagaard et al., 2001).

It has been widely accepted that RT-induced muscle hypertrophy occurs through two primary mechanisms: mechanical tension and metabolic stress (Schoenfeld, 2010, 2013; Pearson and Hussain, 2015). High-load RT (HL-RT, ∼80% of 1-RM) programs seem to simultaneously activate both mechanisms, increasing muscle protein synthesis and, therefore, muscle hypertrophy (Schoenfeld, 2010). On the other hand, low load blood flow restriction resistance training (LL-BFR) produces similar muscle hypertrophy responses to HL-RT, but metabolic stress seems to be the main mechanism inducing the gains in muscle CSA (Takada et al., 2012; Scott et al., 2014), as mechanical tension is usually low (i.e., 20–50% of the 1 RM load). Accordingly, it has been demonstrated that LL-BFR produces greater changes in blood deoxyhemoglobin concentration [HHb] (i.e., proxy marker of metabolic stress) (Cayot et al., 2016; Lauver et al., 2017), compared to traditional low-load RT (Lauver et al., 2017), suggesting higher metabolic stress in the former than in the last (Cayot et al., 2016; Lauver et al., 2017). The high metabolic stress during LL-BFR (e.g., [P_i]) is strongly associated with muscle hypertrophy response after only 2 weeks of training (r = 0.87) (Takada et al., 2012), indicating that mechanical tension may not be required to induce muscle hypertrophy in high metabolic stress conditions. However, we demonstrated that early increases in muscle CSA (i.e., ∼6–9 RT sessions) are partially due to RT-induced muscle damage edema (Damas et al., 2016b #4). Thus, actual muscle hypertrophy response may take longer to be observed (i.e., ∼20 RT sessions) (Damas et al., 2016a, 2018) weakening Takada et al. (2012) findings, and suggesting that further scrutiny is required.

Our group previously demonstrated that high-load blood flow restriction (HL-BFR) training programs do not produce additive effects compared to traditional HL-RT programs (Laurentino et al., 2008). Thus, it is reasonable to suggest that metabolic stress is required to produce muscle hypertrophy only when mechanical tension is low, as in LL-BFR training programs. Thus, assessing [HHb] during HL-BFR and determining its relationship with muscle hypertrophy response may help shedding light into the role of metabolic stress to muscle adaptive response under high mechanical tension.

Therefore, we compared the effects of HL-RT, HL-BFR, and LL-BFR on muscle [HHb], CSA, activation, strength, architecture, and edema before (T1), after 5 (T2), and 10 weeks (T3) of training. Additionally, we analyzed the association between muscle deoxygenation and muscle hypertrophy. Our hypotheses were (1) BFR would not produce an additive effect on neuromuscular adaptations when mechanical tension is high; (2) high mechanical tension protocols (i.e., HL-RT and HL-BFR) would not present an association between metabolic stress and muscle hypertrophy; (3) low load BFR training would produce similar neuromuscular adaptations to HL-RT; and (4) metabolic stress would have an association with muscle hypertrophy only when mechanical tension is low (i.e., LL-BFR).

MATERIALS AND METHODS

Participants

Thirty young men volunteered to participate in the present study (age: 22 ± 3 years; body mass: 72.7 ± 10.7; kg; height: 178 ± 5 cm; BMI: 22.81 ± 2.99 kg·m^2. Inclusion criteria were: (*i*) Not be engaged in regular resistance training and/or endurance training for at least 6 months prior to commencement of the experimental period; (*ii*) being free of cardiovascular and neuromuscular disorders; (*iii*) having a BMI < 30 kg·m^2; and (*iv*) do not use supplements, anti-inflammatory medications and anabolic steroids.

Experimental Design

The present randomized controlled trial used a prospective, single-group, intra-subject design in which each leg of the subjects was exposed to one of three experimental protocols. Before the commencement of the experimental protocol, participants engaged into two familiarization sessions to get acquainted with the training protocol and testing procedures. Familiarization sessions were interspaced by 72 h. Seventy-two hours after the last familiarization session, leg extension 1-RM test was performed. Ninety-six hours after the 1-RM test, vastus lateralis (VL) muscle CSA, echo intensity (CSA_{echo}), pennation angle (PA) were assessed by ultrasonography (US). Each of the participants' legs was allocated to one of the three experimental protocols in a randomized and balanced way according to baseline CSA and leg extension 1-RM values of the dominant leg. In short, participants' legs were divided into quartiles according to muscle CSA and 1-RM values; afterward, legs within each quartile were randomly allocated into the three training protocols: (1) high-load resistance training (HL-RT); (2) high-load resistance training with blood flow restriction (HL-BFR); and (3) low-load resistance training with blood flow restriction (HL-BFR). Importantly, traditional LL-BFR studies have maintained blood flow restricted (i.e., cuff inflated) throughout training sessions. However, maintaining BFR during an exercise session is very uncomfortable and painful (Fitschen et al., 2014) mainly during HL-BFR. Thus, both BFR protocols (LL-BFR and HL-BFR) maintained blood flow restricted only during the exercise (i.e., cuff was deflated during rest intervals) to maintain the level of blood restriction equalized between protocols (Kacin and Strazar, 2011; Grapar Zargi et al., 2016; Zargi et al., 2018). Pilot worked supported our decision as HL-BFR protocol produced higher local deoxygenation than the HL-RT protocol, while maintaining the total training volume (TTV) equalized between the two groups.

The described dependent variables (i.e., CSA, CSA_{echo}/CSA, PA, and 1-RM) were assessed before the experimental protocol (T1), after 5 (T2), and 10 weeks (T3) of the commencement of the experimental period (**Figure 1**). At T2 and T3 all of the measurements were conducted 72 h after the last training bout performed. Muscle activation and oxygenation were assessed by

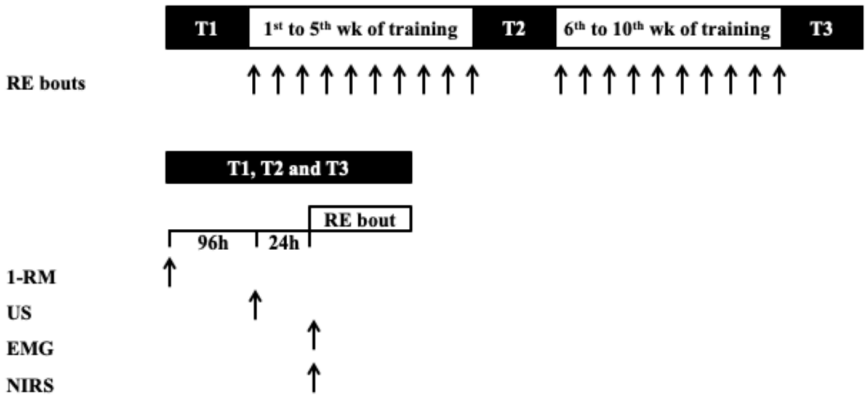

FIGURE 1 | Schematic of study protocol. RE, resistance exercise; 1-RM, one-repetition maximum; EMG, electromyographic; NIRS, near-infrared spectroscopy.

EMG and near infrared spectroscopy (NIRS), respectively, during a training session in T1, T2, and T3, where each leg performed the training protocol according to the initial randomization.

Maximum Dynamic Strength Test

Unilateral quadriceps maximum dynamic strength was assessed using the 1-RM test on a leg-extension machine (Effort NKR; Nakagym, São Paulo, Brazil), according to the procedures described elsewhere (Brown and Weir, 2001). In short, participants performed a general warm-up on a cycle ergometer at 20 $km \cdot h^{-1}$ for 5 min, followed by specific warm-up sets of leg-extension exercise. In the first set, individuals performed 8 repetitions with a load corresponding to 50% of their estimated 1-RM, obtained during the familiarization sessions. In the second set, they performed three repetitions at 70% of their estimated 1-RM. A 2 min interval was allowed between warm-up sets. After the last warm-up set, a 3 min resting period before the actual 1-RM test. Participants had up to five attempts to achieve their 1-RM load. The smallest increment in load on subsequent attempts was of approximately one kilogram. A 3 min rest interval was allowed between attempts and the highest load achieved (full eccentric–concentric movement with 90° range of motion) was considered as the 1-RM load. The coefficient of variation (CV) and typical error (TE) between two repeated measurements performed 72 h were 1.57% e 1.45 Kg, respectively.

Determination of the Blood-Flow Restriction Pressure

Before the commencement of the training protocol, blood-flow restriction pressure was determined as follows. Participants in the HL-BFR and LL-BFR protocols were asked to rest comfortably in supine position. A vascular Doppler probe (DV-600; Marted, Ribeirão Preto, São Paulo, Brazil) was placed over the tibial artery to capture its auscultatory pulse. For the determination of blood pressure (mmHg) necessary for complete vascular occlusion (pulse elimination pressure), a standard blood-pressure cuff [175 mm (width) 920 mm (length)] was wrapped around the participant's thigh at the inguinal fold region and then inflated up to the point at which the auscultatory pulse was interrupted (Libardi et al., 2015a). The pressure in which the auscultatory pulse was interrupted was considered as the occlusion pressure.

Muscle Cross-Sectional Area

A B-mode ultrasound (US) with a 7.5-MHz linear-array probe (Mysono U6 EX; Samsung-Medison, Gangwon-do, South Korea) was used to capture images in the axial plane of the VL muscle after the participants had laid supine for 20 min to allow for fluid distribution before the assessments (Berg et al., 1993). The image reconstruction technique was used to assess VL muscle CSA following procedures described by our group Lixandrao et al. (2014). In short, the legs were restrained with Velcro® straps to avoid movements of the lower limbs while allowing the participants to relax the leg muscles during the assessments. All the US images were obtained at midpoint between the inferior border of the lateral epicondyle and the greater trochanter of the femur. To increase the accuracy of the measurements at T1, T2, and T3, the skin was marked with semi-permanent ink every 2 cm from the medial to the lateral aspect of the vastus lateralis muscle. Sequential US images were acquired aligning the superior edge of the probe with each of the 2 cm mark, following a middle-to-lateral direction. To avoid deforming the tissue with the pressure applied on the US probe, a generous amount of conductive gel was used. Sequential images of the vastus lateralis muscle were opened in PowerPoint (Microsoft, Redmond, WA, United States), and then each image was manually rotated to reconstruct the whole fascia of the VL muscle. Subsequently, the VL muscle CSA was measured using computerized planimetry, in which the VL muscle CSA was contoured following the muscle fascia using an 800 dpi mouse (Madena 3.2.5; Eye Physics, Los Alamitos, CA, United States). The CV and the TE between two repeated measurements with an interval of 72 h were 0.99% and 0.24 cm^2, respectively.

Muscle Cross-Sectional Area Echo Intensity (CSA_{echo})

Muscle CSA previously delimited was analyzed using a Fast Fourier transformation to identify the frequency spectrum of the pixel intensity over the VL CSA (CSA_{echo}). This analysis results

in a histogram of grayscale shades (0 = black and 256 = white), where any abnormality (e.g., edema-induced muscle swelling, probably due to muscle damage) results in higher echo intensity value (increased white areas), while intact muscle mass presents low echo intensity (i.e., dark areas) (Scanlon et al., 2013; Damas et al., 2016b). Importantly, this method has been extensively used as an indicator of exercise-induced muscle damage edema (Nosaka and Sakamoto, 2001; Chen and Nosaka, 2006; Cheng et al., 2009; Chen et al., 2012, 2013; Gonzalez-Izal et al., 2014; Rosenberg et al., 2014; Damas et al., 2016b). CSA_{echo} was normalized by the vastus lateralis CSA (i.e., CSA_{echo}/CSA) to account for the effects of changes in muscle size.

Pennation Angle (PA)

PA of the VL was measured using the B-mode ultrasound at the thigh mid-point. The PA was defined as the angle between the fascicle and the deep aponeurosis of the VL muscle (**Table 1**) (Fukunaga et al., 1997). Three consecutive images were obtained and the average value of the PAs was considered for statistical purposes (Blazevich et al., 2007; Alegre et al., 2014; Ando et al., 2014). PA CV and TE between two repeated measurements performed 72 h were 0.035% and 0.670°, respectively.

Knee Joint Angle and Trigger

An angular potentiometer was placed on the right knee of the individuals with its center of rotation aligned with the lateral intercondylar line of the knee joint to determine knee angular excursion and, therefore, the concentric and eccentric phases of the lift. Full extension was defined as "zero degree." The concentric phase was defined from the maximum to the minimum value of the knee flexion angle, while the eccentric phase was defined from the minimum to the maximum value of the knee flexion angle. The frequency of acquisition was set at 1000 Hz in the A/D converter of the EMG unit described below, which synchronized data acquisition from the angular potentiometer and both the EMG system and the NIRs device. The signal from an external trigger was split and sent to both EMG and NIRs A/D converters to align the data in time.

TABLE 1 | Muscle cross section area (CSA), CSA echo intensity (CSAecho), CSAecho to CSA ratio, pennation angle (PA) and maximum dynamic strength test (1-RM) at baseline (T1), after 5 (T2), and 10 weeks (T3) for high-load resistance training (HL-RT), high-load resistance training with blood flow restriction (HL-BFR) and low-load strength training with blood flow restriction (LL-BFR).

Variable	Time	HL-RT	HL-BFR	LL-BFR
CSA (cm^2)	T1	22.3 ± 6.7	21.8 ± 4.0	21.4 ± 5.8
	T2*	23.4 ± 7.0	22.9 ± 4.3	22.6 ± 6.2
	T3*†	24.5 ± 7.4	24.2 ± 4.7	23.7 ± 6.7
CSA_{Echo} (AU)	T1	19.5 ± 5.1	19.5 ± 5.5	21.0 ± 7.0
	T2*	38.0 ± 9.0	38.2 ± 9.7	40.0 ± 11.0
	T3†	17.6 ± 3.3	19. ± 5.5	17.6 ± 4.1
CSA_{Echo} (AU)/CSA (cm^2)	T1	1.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.4
	T2*	1.7 ± 0.6	1.7 ± 0.4	2.0 ± 0.9
	T3*†	0.7 ± 0.2	0.8 ± 0.3	0.8 ± 0.2
PA (°)	T1	15.0 ± 2.6	15.4 ± 2.0	14.6 ± 2
	T2*	16.0 ± 2.8	16.3 ± 2.0	15.5 ± 2.0
	T3*†	16.4 ± 2.9	17.0 ± 2.1	16.0 ± 2.1
1-RM (kg)	T1	46.6 ± 11.5	46.4 ± 10.8	46.5 ± 11.7
	T2*	55.7 ± 10.8	56.5 ± 10.4	51.3 ± 17.7
	T3*†	63.8 ± 10.9	62.1 ± 18.1	55.2 ± 23.2

*Significantly different from T1 (main time effect, $P < 0.05$), †Significantly different from T2 (main time effect, $P < 0.05$). Values presented as mean ± SD.

Muscle Activation

Muscle activation of the VL muscle, assessed by the amplitude of the electromyographic (EMG) signal (EMG832C; EMG System do Brazil, São José dos Campos, Brazil), was determined at T1, T2, and T3. Before electrode placement, the skin area was shaved, abraded and cleaned with an isopropyl alcohol pad to reduce skin impedance before electrode placement (Libardi et al., 2015b). Pre-gelled Ag/Ag-CL surface electrodes (EMG System, São José dos Campos, Brazil) were placed over the belly of the VL muscle aligned in parallel with the expected muscle fiber orientation and with an interelectrode distance of 2 cm. In addition, a ground electrode was placed in the ankle region at the fibular lateral malleolus. The sampling frequency of the EMG signals was of 1000 Hz with a band-pass filter of 20 and 500 Hz. The EMG amplifiers have an input noise below 1 μV root mean square (RMS) and an effective common rejection mode of 95 dB. Electromyography RMS values were calculated over the concentric phase, defined as the maximum and minimum knee joint angle, on each repetition. Then, RMS values were normalized by the maximal muscle activation (i.e., RMS) obtained during a maximal voluntary isometric contraction (MVIC), calculated over a 250 ms interval around peak torque. After visual inspection, RMS values calculated on each repetition of all training sets (i.e., 1st, 2nd, and 3rd sets) were numerically integrated over time using the trapezoidal rule (GraphPad Prism, GraphPad Software, San Diego, CA, United States), and used for further analysis. The MVIC torque was measured before the exercise protocol having a load cell attached at 90° with the lever arm of the leg extension machine. Torque was calculated by the product of the force values by the length of the shank (i.e., distance from the lateral intercondilar line to the lateral malleolus). Load cell data was acquired at a frequency of 1000 Hz using the A/D converter of the EMG unit and digitally filtered with a Butterworth filter set a low pass frequency of 20 Hz. The normalized results of EMG (%MVIC) were presented as mean values of over the concentric phases of the three sets performed during the exercise sessions at T1, T2, and T3.

Muscle Oxygenation

A continuous dual-wavelength near-infrared spectroscopy apparatus (NIRS; Oxymon, Artinis Medical Systems, Arnhem, the Netherlands) was used to monitor changes in muscle oxygenation during the HL-RT, HL-BFR, and LL-BFR protocols in T1, T2, and T3 assessments. Data was collected at a frequency of 25 Hz. The system uses a modified Beer-Lambert law to analyze the changes in light absorbed at wave lengths of 761 and 844 nm, estimating concentrations of deoxygenated hemoglobin ([HHb]), which has been considered an indicator of metabolic stress (Cayot et al., 2016; Lauver et al., 2017).

Initially, thickness of the subcutaneous fat layer at the site of NIRs optodes (i.e., an emitter and a detector) placement was assessed by ultrasound (HL: 0.83 ± 0.18 cm, HL-BFR: 0.91 ± 0.22 cm, and LL-BFR: 0.87 ± 0.20 cm) to set the value of laser penetration depth (Koga et al., 2007). Following, a large area of skin was shaved and cleaned with alcohol. Then, the holder of the pair of optodes was fixed on the skin with adhesive tapes. The optodes were positioned in the VL muscle, 3 cm medial from the point used to fix EMG electrodes (Ferrari et al., 2004). Before each data collection, the equipment was set to a sampling frequency of 25 Hz (Kacin and Strazar, 2011).

Data were extracted from the NIRS device using Oxisoft (3.0.X; Artinis Medical Systems B.V, Arhem, Netherlands) and a customized script analyzed the data off-line. Raw data was filtered with a moving average algorithm over a 2 s period. Then, [HHb] resting values were obtained during the last 5 min of a 15 min rest period, in which individuals remained still and as relaxed as possible prior to exercise commencement (Tanimoto and Ishii, 2006; Kon et al., 2010; Callewaert et al., 2013). Exercise values were determined as the difference between the [HHb] values, obtained over the concentric phase of each repetition (as described above), and the mean [HHb] value over the 5 min resting period. [HHb] values calculated on each repetition of all training sets (i.e., 1st, 2nd, and 3rd sets) were numerically integrated over time using the trapezoidal rule (GraphPad Prism, GraphPad Software, San Diego, CA, United States), for each of the assessments (i.e., T1, T2, and T3) (Ganesan et al., 2015), and used for further analysis.

Resistance Training Protocols

Training protocols were performed unilaterally using a conventional leg-extension machine, twice a week for 10 weeks. The HL-RT and HL-BFR protocols performed 3 sets of 10 repetitions with a load corresponding to 80% 1-RM (HL-RT: T1–T2 = 37.2 ± 9.2 kg and T2–T3 = 44.5 ± 8.6 kg; HL-BFR: T1–T2 = 37.1 ± 8.7 kg and T2–T3 = 45.2 ± 8.4 kg), while the LL-BFR protocol performed 3 sets of 20 repetitions with 20% 1-RM (LL-BFR: T1–T2 = 9.3 ± 2.3 kg and T2–T3 = 10.2 ± 3.5 kg). A 1 min rest period was granted between sets for all of the protocols. After the fifth week (10th session), 1-RM was re-assessed to adjust training load. From week 6 (T2–T3) onwards, the number of sets was increased to four, for all of the participants. The cuff pressure used during the BFR protocols was set at 60% of occlusion pressure in the resting condition. The cuff pressure remained inflated during the exercise and deflated during the rest periods. The average pressure used throughout the training protocol was 81.85 ± 4.45 mmHg.

Statistical Analysis

After visual inspection, the area under the curve (AUC) analysis for EMG and [HHb] were performed using the trapezium rule (GraphPad Prism, GraphPad Software, San Diego, CA, United States) in order to characterize the magnitude of the response and the changes over time. AUC analyses were calculated using the time point immediately before (Pre) and changes in EMG and [HHb] in the 1st, 2nd, and 3rd sets. The 1-RM, CSA, CSAecho, CSAecho/CSA, PA, EMG, and [HHb] data were analyzed using mixed models having training protocol and time as fixed factors, and subjects as random factor. Only TTV was analyzed with a one-way repeated measures model having training protocol as a fixed factor and subjects as a random factor. In case of significant values of F, a Fisher's LSD *post hoc* analysis was used for multi comparison purposes. Pearson correlation was used to estimate the association between changes in [HHb] (Average of the values of T1 and T2, T2 and T3, T1 and T3 multiplied by the number of sessions in the same periods) and muscle CSA (Percentage change from T1 to T2, T2 to T3, and T1 to T3). All statistical analyses were performed using SAS software (SAS Institute Inc, Cary, NC, United States). Effect sizes (ES) were calculated for 1-RM and muscle CSA using the changes from T1 to T3. ES were classified as "small" if lower than 0.2, "medium" if between 0.2 and 0.5, and "large" if higher than 0.8 (Cohen, 1988). Significance level was set at $P < 0.05$ and data was presented as mean and standard deviation (SD).

RESULTS

Total Training Volume (TTV)

TTV (sets × repetitions × load [kg]) in the LL-BFR (11733.0 ± 3204.9 kg) was lower than HL-RT (24546.0 ± 5329.0 kg, $P < 0.0001$) and HL-BFR (24708.0 ± 4992.4 kg; $P < 0.0001$) protocols. No significant differences in TTV were detected between the HL-RT and HL-BFR protocols ($P > 0.05$).

Maximum Dynamic Strength Test (1-RM)

1-RM values increased, similarly, and significantly for the HL-RT, HL-BFR, and LL-BFR groups from T1 to T2 (main time effect $P < 0.0001$) and from T1 to T3 [ES: HL-RT, 1.24 (large); HL-BFR, 1.42 (large) and LL-BFR, 0.96 (large); main time effect, $P < 0.0001$]. In addition, 1-RM values at T3 were significantly greater than that at T2 (main time effect $P < 0.0001$) (**Table 1**).

Muscle Cross-Sectional Area (CSA)

The HL-RT, HL-BFR, and LL-BFR protocols groups showed significant and similar increases in muscle CSA from T1 to T2 (main time effect, $P < 0.0001$) and from T1 to T3 [ES: HL-RT, 0.26 (moderate); HL-BFR, 0.46 (moderate), and LL-BFR, 0.30 (moderate); main time effect, $P < 0.0001$]. There was also a significant increase from T2 to T3 (main time effect, $P < 0.0001$) (**Table 1**).

CSA Echo Intensity (CSA_{echo})

CSA_{echo} analysis (**Table 1**) showed significant increases from T1 to T2 (main time effect, $P < 0.0001$), and significant decreases from T2 to T3 (main time effect, $P < 0.0001$). There were no significant changes in CSA_{echo} from T1 to T3 ($P > 0.05$). When CSA_{echo} was normalized by VL CSA (CSA_{Echo}/CSA), T2 was significantly elevated in all three conditions compared to T1 (main time effect, $P < 0.0001$) and T3 (main time effect $P < 0.0001$). In addition, T3 showed significantly lower values than T1 for the three protocols (main time effect $P < 0.0001$).

Pennation Angle (PA)

In relation to the PA (**Table 1**), HL-RT, HL-BFR, and LL-BFR protocols produced significant increases from T1 to T2 (main time effect, $P < 0.0001$) and from T1 to T3 (main time effect $P < 0.0001$). There were also significant increases from T2 to T3 for all groups (main time effect, $P < 0.0001$).

Muscle Activation

EMG amplitude AUC was greater during HL-RT and HL-BFR than LL-BFR (main protocol effect, $P = 0.01$ and $P = 0.001$), with no differences between HL-RT and HL-BFR ($P > 0.05$) (**Figure 2**). Additionally, EMG amplitude AUC was not different between T1, T2, and T3 ($P > 0.05$) (**Figure 2**).

Muscle Oxygenation

The [HHb] AUC was significantly lower for all protocols in T3 compared to T2 (main time effect, $P = 0.04$), but similar to T1. No differences were observed between T1 and T2 ($P > 0.05$). Regarding protocol comparison, [HHb] AUC was greater during HL-BFR and LL-BFR than HL-RT (main protocol effect, $P = 0.02$ and $P = 0.03$), with no difference between HL-BFR and LL-BFR ($P > 0.05$) (**Figure 3**).

There was a significant correlation between [HHb] and CSA area only at T2–T3 for the LL-BFR protocol ($r = 0.71$; $P = 0.0008$) (**Table 2**). No significant correlations were found between [HHb] and CSA for the HL-RT and HL-BFR protocols ($P > 0.05$). Additionally, we collapsed the groups to test overall significant correlations between muscle oxygenation variables and hypertrophy response and no significant correlations were observed ($P > 0.05$).

DISCUSSION

We aimed to compare the effects of HL-RT, HL-BFR, and LL-BFR on muscle deoxygenation (HHb), CSA, activation, strength, architecture, and edema before (T1), after 5 (T2), and 10 weeks (T3) of training. Additionally, we analyzed the association between [HHb] and muscle hypertrophy. Regarding our four hypotheses, we confirmed that: (1) BFR did not produce an additive effect on muscle hypertrophy when mechanical tension is high; (2) high mechanical tension protocols did not produce a significant correlation between metabolic stress and muscle hypertrophy (i.e., HL-RT and HL-BFR); (3) LL-BFR produced similar neuromuscular adaptations to HL-RT; and (4) metabolic stress has a positive and significant association with muscle hypertrophy only when mechanical tension is low (i.e., LL-BFR). Thus, mechanical tension and metabolic stress seem to share the variance of the muscle hypertrophy response under high mechanical tension protocols, while metabolic stress seems to be the main mechanism responsible for muscle hypertrophy when mechanical tension is low.

Muscle Strength

Regarding muscle strength, previous studies have reported similar increases in muscle strength between HL-RT and LL-BFR protocols (Takarada et al., 2000; Karabulut et al., 2010; Laurentino et al., 2012). For instance, Laurentino et al. (2012) demonstrated significant and similar increases in 1-RM values after 12 weeks in HL-RT and LL-BFR protocols (36.2 and 40.1%, respectively). In our study, muscle strength increased significantly after 10 weeks of training (HL-RT = 41.0% and LL-BFR = 32.2%, respectively). Thus, the increases in muscle strength after LL-BFR protocol are comparable to previous studies (Laurentino et al., 2012). Increments in muscle strength in LL-BFR protocols are usually attributed to the maintenance of BFR throughout the training session (exercise and pause between sets). The findings reported herein show otherwise, as we deflated the BFR cuff during the resting intervals. Furthermore, our findings support previous ones (Laurentino et al., 2008) as BFR did not produce additive effects on strength gains when mechanical tension was high (i.e., HL-BFR). Taken together, these findings are of great importance for the viability of LL-BFR protocols, as when BFR is applied only during exercise, it promotes lower perception of pain compared to traditional BFR protocols (Fitschen et al., 2014).

Muscle Hypertrophy

Usually, studies have reported that low-load RT promotes a small or even no increase in the muscle CSA (Takarada et al., 2000; Yasuda et al., 2010; Laurentino et al., 2012). However, our group and others (Takarada et al., 2002; Laurentino et al., 2012; Yasuda et al., 2012, 2013; Vechin et al., 2014; Libardi et al., 2015a; Lixandrao et al., 2015) have shown that the addition of BFR to low-load RT produces increases in muscle CSA (between 6 and 7.5%), comparable to HL-RT (between 6 and8%), after a 12 weeks training. In our study, we found significant and similar increases in CSA after 5 and 10 weeks of HL-RT (4.9 and 10.0%, respectively) and LL-BFR (5.0 and 10.0%, respectively). Despite the release of BFR cuff pressure during the rest interval between sets, our results suggest that muscle hypertrophy response is not affected when compared to LL-BFR protocols in which BFR cuff is maintained inflated throughout the training session. Regarding the comparison between HL-RT and HL-BFR, our results demonstrated that the addition of BFR does not result in additional increases in muscle CSA, as these protocols presented virtually the same changes in VL CSA at T2 (4.98 and 5.19%, respectively) and T3 (10.01 and 10.36%, respectively) compared to T1. Although only one study has investigated the effects of HL-BFR, Laurentino et al. (2008) reported similar increases in quadriceps CSA for HL-RT and HL-BFR after 8 weeks of training (6.1 and 5.0%, respectively).

Importantly, muscle hypertrophy was accompanied by increases in pennation angle (PA). These increases occurred in the early stages of training [T2, 5 weeks (10 training sessions)] and were even greater at T3 [10 weeks (20 training sessions)], as shown in other studies (Seynnes et al., 2007). However, in the present study, the increases in CSA and PA were accompanied by higher CSA_{echo} in T2 than T1, followed by a reduction in T3. These results indicate that muscle hypertrophy and changes in muscle architecture observed in T2 were likely related to edema/muscle damage, and not to the expansion of myofibrillar content. Recently, our group demonstrated that after 2 weeks of RT, increases in muscle CSA were accompanied by enhanced

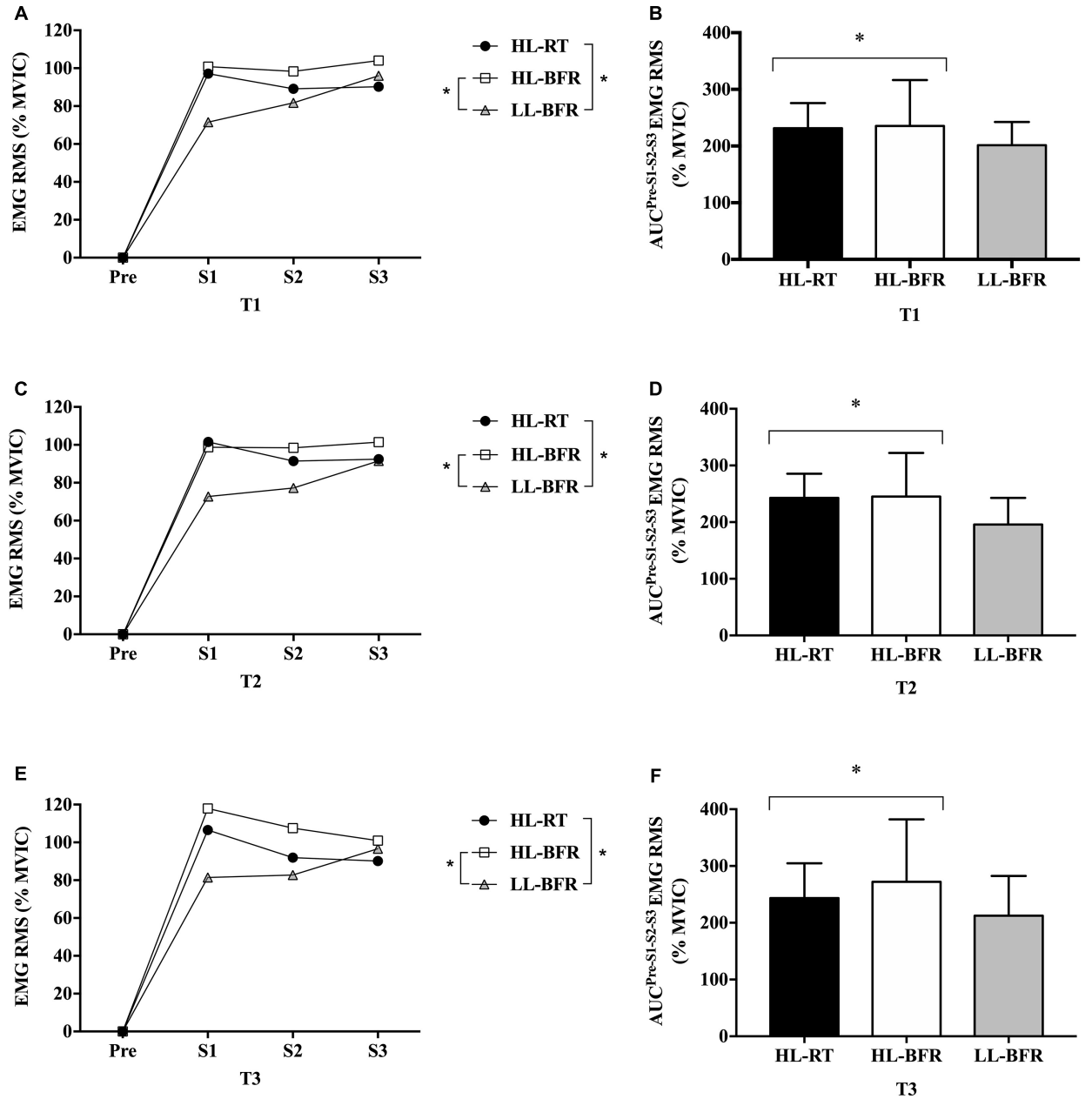

FIGURE 2 | Normalized electromyographic (EMG) root mean square (RMS) values from the resistance training session (Pre [Before the beginning of the exercise], set 1 [S1], set 2 [S2], and set 3 [S3]) at baseline (T1, **A**), after 5 (T2, **C**) and 10 weeks (T3, **E**) for the high-load resistance training (HL-RT), high-load resistance training with blood flow restriction (HL-BFR) and low-load resistance training with blood flow restriction (LL-BFR) protocols. EMG RMS values also are reported as area under the curve (AUC) from the entire resistance training session at T1 **(B)**, T2 **(D)**, and T3 **(F)** for the HL-RT, HL-BFR and LL-BFR protocols. *Significantly different from LL-BFR (main protocol effect, $P < 0.01$). Values presented as mean ± SD.

CSA_{echo} and systemic markers of muscle damage (e.g., myoglobin and interleukin-6) (Damas et al., 2016b). Accordingly, it has been shown that only a single HL-RT or LL-BFR bout can change the muscle architecture due to edema caused by exercise-induced muscle damage (Kubo et al., 2006; Martin-Hernandez et al., 2013; Yu et al., 2015). To support this idea, we normalized the CSA_{echo} by CSA (i.e., CSA_{Echo}/CSA) and the results demonstrated that T2 values were also significantly higher than T1 and T3 ones. Supporting our previous findings (Damas et al., 2016b), we observed a significant decrease in CSA_{echo} with concomitant increases in CSA and PA at T3. These results suggest that after 20 training sessions (i.e., 10 weeks), muscle damage-related changes in muscle morphology are attenuated, and muscle hypertrophy produced the changes observed in morphology. Furthermore, the strong association between changes in intramuscular [Pi] (i.e., metabolic stress marker) and muscle CSA ($r = 0.87$), reported by

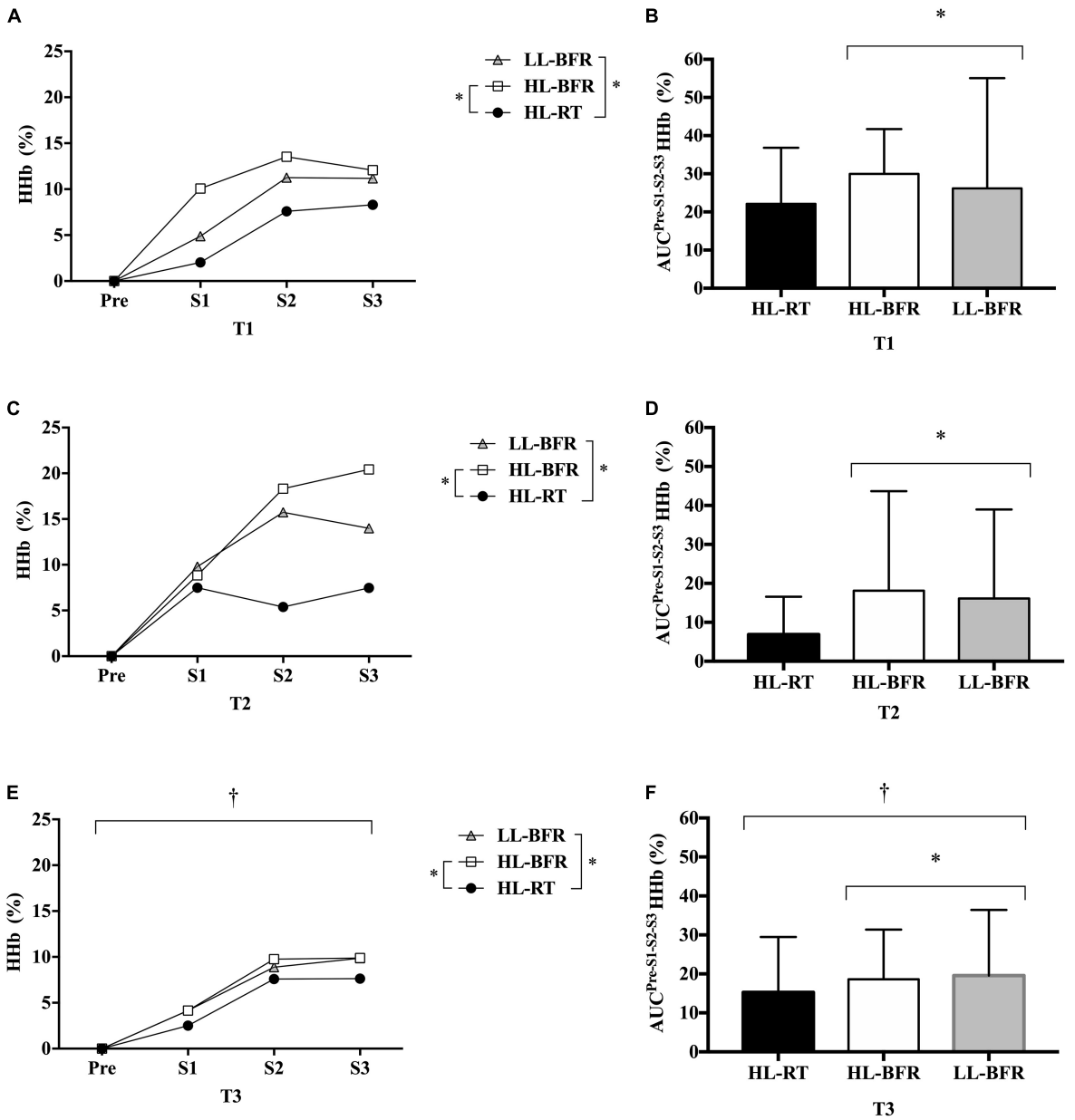

FIGURE 3 | Normalized deoxygenated hemoglobin ([HHb]) values from the resistance training session (Pre [Before the beginning of the exercise], set 1 [S1], set 2 [S2], and set 3 [S3]) at baseline (T1, **A**), after 5 (T2, **C**) and 10 weeks (T3, **E**) for the high-load resistance training (HL-RT), high-load resistance training with blood flow restriction (HL-BFR) and low-load resistance training with blood flow restriction (LL-BFR) protocols. [HHb] values also are reported as area under the curves (AUC) from the entire resistance training session at T1 **(B)**, T2 **(D)**, and T3 **(F)** for the HL-RT, HL-BFR, and LL-BFR protocols. *Significantly different from HL-RT (main protocol effect, $P < 0.04$). †Significant different from T2 (main time effect, $P < 0.04$). Values presented as mean ± SD.

Takada et al. (2012) may have been induced by muscle edema, rather than actual muscle hypertrophy, as it artificially increases muscle CSA (Damas et al., 2016b).

Muscle Activation

It has been suggested that muscle strength gains and hypertrophy after a RT period, with or without BFR, are associated with increases in the ability to activate the motor unit pool (Suga et al., 2012; Schoenfeld, 2013) as assessed by the amplitude of the surface EMG signal (Takarada et al., 2000; Laurentino et al., 2008; Sundstrup et al., 2012). However, few studies have investigated the changes in EMG amplitude during the actual training protocol (Kacin and Strazar, 2011). In the present study, we observed higher EMG amplitude in T1, T2, and T3 for HL-RT and HL-BFR compared to LL-BFR. Considering the comparison between HL-RT and LL-BFR, some acute studies (i.e., a single training session) also showed that EMG values were higher for HL-RT compared to LL-BFR (Kubo et al., 2006; Manini and

TABLE 2 | Correlations between changes in deoxyhemoglobin concentrations ([HHb]) and changes in muscle cross-sectional area (CSA) for high-load resistance training (HL-RT), high-load resistance training with blood flow restriction (HL-BFR), low-load blood flow restriction (LL-BFR) and all groups together.

Variable		CSA (%) (T1–T2)	CSA (%) (T2–T3)	CSA (%) (T1–T3)
HL-RT [HHb] (T1–T2)	*r*	0.30		
	P	0.19		
HL-RT [HHb] (T2–T3)	*r*		0.14	
	P		0.56	
HL-RT [HHb] (T1–T3)	*r*			0.03
	P			0.90
HL-BFR [HHb] (T1–T2)	*r*	0.136		
	P	0.580		
HL-BFR [HHb] (T2–T3)	*r*		0.43	
	P		0.08	
HL-BFR [HHb] (T1–T3)	*r*			0.05
	P			0.86
LL-BFR [HHb] (T1–T2)	*r*	0.10		
	P	0.67		
LL-BFR [HHb] (T2–T3)	*r*		0.71	
	P		0.0008*	
LL-BFR [HHb] (T1–T3)	*r*			0.36
	P			0.22
All protocols [HHb] (T1-T2)	*r*	0.05		
	P	0.71		
All protocols [HHb] (T2–T3)	*r*		0.09	
	P		0.23	
All protocols [HHb] (T1–T3)	*r*			0.05
	P			0.75

**Significant correlation ($P < 0.05$).*

Clark, 2009). Regarding HL-RT and HL-BFR, to our knowledge, only one study compared the EMG amplitude between HL-RT and HL-BFR, and found no significant differences between these protocols after a single bout (Neto et al., 2014). Collectively, these results suggest that the recruitment of MUs does not seem to explain the similarity in muscle strength and hypertrophy gains between these protocols. Limitations of the EMG assessment (Dimitrova and Dimitrov, 2003) and other possible mechanisms may have contributed to these findings.

Muscle Oxygenation

It has been suggested that [HHb] is a proxy marker of metabolic stress (Cayot et al., 2016; Lauver et al., 2017), which has been considered as a primary stimulus to muscle growth (Schoenfeld, 2010, 2013; Pearson and Hussain, 2015). Accordingly, studies have used BFR during low-load RT to increase metabolic stress, and to induce muscle hypertrophy response (Takada et al., 2012). However, little is known regarding the role of metabolic stress in high mechanical tension (e.g., HL-RT) protocols and if increases in metabolic stress may produce and additive effect on muscle hypertrophy response. In this study, we added BFR to both low- and high-load RT and compared them with HL-RT, traditionally recommended to maximize increases in muscle strength and hypertrophy (American College of Sports Medicine [ACSM] , 2009, 2011). Metabolic stress can be measured by near-infrared spectroscopy (NIRS) during exercise, as it non-invasively identifies changes in the relative concentrations of deoxyhemoglobin ([HHb]) in muscle tissue. Regarding, the comparison between time points, we observed that after 10 weeks (T3) of training, increases in [HHb] were lower than at T2 (5 weeks) and similar to T1 for all protocols. In this sense, Kacin and Strazar (2011) investigated the effects of 4 weeks of low-load RT with and without BFR during knee-extension exercise at 15% maximal voluntary muscle contraction to the voluntary failure. The results show attenuated decrease in concentrations of oxygenated hemoglobin ($[HbO_2]$) after the training period for both experimental conditions. Although studies have observed changes in different markers throughout the RT, both suggest a peripheral adaptation probably induced by local angiogenesis responses to RT protocols. Indeed, RT protocols might increase vascular endothelial growth factor expression and other transcription factors/growth associated with the formation of capillaries (i.e., angiogenesis) (Gavin et al., 2007).

Comparing the protocols, those performed with BFR (i.e., HL-BFR and LL-BFR) showed higher deoxygenation compared to HL-RT. Interestingly, notwithstanding HL-BFR combine the highest levels of metabolic stress and mechanical tension, the muscle hypertrophy and strength gains were similar to protocols with lower levels of metabolic stress (i.e., HL-RT) or mechanical tension (i.e., LL-BFR). These results may suggest that muscle protein synthesis may reach maximal values when training with high intensities (e.g., 80% 1-RM – high mechanical tension) and the metabolic stress does not seem to produce additive effects to muscle hypertrophy. On the other hand, in low-load protocols (e.g., 30% 1-RM) the metabolic stress induced by BFR seems to fully activate the muscle protein synthesis machinery. This is supported by the strong association between changes in [HHb] and changes in muscle CSA only to LL-BFR group ($P = 0.008$; $r = 0.716$). Further studies are required to elucidate this issue.

Limitations

(1) We show greater muscle deoxygenation for HL-BFR and LL-BFR and higher muscle activation for HL-RT and HL-BFR, but with similar neuromuscular adaptations between these protocols in untrained men. Although one may rightly suggest that these findings may not be directly extended to trained individuals, to the best of our knowledge, there is no empirical evidence suggesting that the activation of specific hypertrophy triggers may change as a function of the training status.

(2) A high and significant correlation between [HHb] and muscle hypertrophy were showed for LL-BFR. However,

these findings should be viewed with caution, as other NIRS parameters, such as deoxyhemoglobin (HbO_2), total hemoglobin (HBT) and hemoglobin difference (Hbdiff) were not analyzed in the present study. In addition, the agreement between [HHb] and other parameters related to metabolic stress (e.g., phosphocreatine, inorganic phosphate, muscle pH, and lactate) should be analyzed in future studies to confirm our findings. On the other hand, the HHb is highly correlated with blood lactate (Grassi et al., 2003), which has been traditionally considered as a proxy marker of metabolic stress (Cayot et al., 2016; Lauver et al., 2017). Furthermore, it has been previously reported that the [HHb] signal is less sensitive to changes in blood volume compared to other markers related to muscle oxygenation (Ferrari et al., 1997). Thus, [HHb] seems to be a better index to compare protocols with BFR, as they may produce large fluctuations in blood volume.

(3) We also suggest that future studies should investigate the effects of secondary hypertrophy-related mechanisms triggered by metabolic stress (e.g., increased fast-twitch fiber recruitment, local hormone, cell swelling, and the production of reactive oxygen species) on muscle hypertrophy to further elucidate these complex mechanisms.

(4) It is also important to consider that the findings reported herein should be confirmed in other BFR training protocols, as it has been demonstrated that metabolic stress may change as a function of the occlusion pressure and training load (Suga et al., 2010).

(5) BFR training pressure was determined in a resting state, which may be considered as an inherited limitation of the method, as pressure fluctuates during the eccentric and concentric phases of the lifts.

Physiological Relevance

Muscle deoxygenation seems to play an important role on neuromuscular adaptations when RT is performed with low-load, as it produces similar neuromuscular adaptions to high-load protocols, despite the lower (TTV) (∼53%). Corroborating this suggestion, there was a significant association between the changes in [HHb] and increases in muscle CSA from T2 to T3, when muscle edema was attenuated. We propose that the level of metabolic stress would not influence the magnitude of muscle hypertrophy, as well as changes in muscle strength and architecture, when RT is performed in high-loads.

CONCLUSION

Our results suggest that the addition of BFR to exercise contributes to neuromuscular adaptations only when RT is performed with low-load. Furthermore, we found a significant association between the changes in [HHb] (i.e., metabolic stress) and increases in muscle CSA from T2 to T3 only for the LL-BFR, when muscle edema was attenuated.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of ethics committee of Federal University of São Carlos (UFSCar), number 42359015.5.0000.5504 with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee of Federal University of São Carlos (UFSCar).

AUTHOR CONTRIBUTIONS

CAL had the original idea of the study and the final study design was developed by CAL, CU, TMPCB, and AB-S. Participants were recruited, trained and assessed at the Federal University of São Carlos, by TMPCB, RMO, SDS, and JGB. TMPCB, CAL, and CU performed data analyses and statistical procedures and wrote the first version of the manuscript. All authors participated in the interpretation of the data, contributed to the revision of the manuscript, and approved the content of the final version.

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Blood Flow Restriction Exercise Attenuates the Exercise-Induced Endothelial Progenitor Cell Response in Healthy, Young Men

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Endothelial progenitor cells (EPCs) are a vasculogenic subset of progenitors, which play a key role in maintenance of endothelial integrity. These cells are exercise-responsive, and thus exercise may play a key role in vascular repair and maintenance via mobilization of such cells. Blood flow restriction exercise, due to the augmentation of local tissue hypoxia, may promote exercise-induced EPC mobilization. Nine, healthy, young (18–30 years) males participated in the study. Participants undertook 2 trials of single leg knee extensor (KE) exercise, at 60% of thigh occlusion pressure (4 sets at 30% maximal torque) (blood flow restriction; BFR) or non- blood flow restriction (non-BFR), in a fasted state. Blood was taken prior, immediately after, and 30 min after exercise. Blood was used for the quantification of hematopoietic progenitor cells (HPCs: $CD34^{+}CD45^{dim}$), EPCs ($CD34^{+}VEGFR2^{+}$/$CD34^{+}CD45^{dim}VEGFR2^{+}$) by flow cytometry. Our results show that unilateral KE exercise did not affect circulating HPC levels (p = 0.856), but did result in increases in both $CD34^{+}VEGFR2^{+}$ and $CD34^{+}CD45^{dim}VEGFR2^{+}$ EPCs, but only in the non-BFR trial ($CD34^{+}VEGFR2^{+}$: 269 ± 42 cells mL^{-1} to 573 ± 90 cells mL^{-1}, pre- to immediately post-exercise, p = 0.008; $CD34^{+}CD45^{dim}VEGFR2^{+}$: 129 ± 21 cells mL^{-1} to 313 ± 103 cells mL^{-1}, pre- to 30 min post-exercise, p = 0.010). In conclusion, low load BFR exercise did not result in significant circulating changes in EPCs in the post-exercise recovery period and may impair exercise-induced EPC mobilization compared to non-BFR exercise.

Keywords: endothelial progenitors, exercise, endothelial, angiogenesis, blood flow restricted exercise

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INTRODUCTION

Endothelial progenitor cells (EPCs) were first discovered in 1997 by Asahara et al. (1997). These peripheral blood mononuclear cells (PBMC) could form endothelial cell-like networks and differentiate into mature endothelial cell phenotypes *in vitro*. These cells are bone marrow-derived and can be mobilized in response to vascular injury or an inflammatory stimulus (Asahara et al., 1999; Shintani et al., 2001). Since 1997, there have been a plethora of studies reporting their vasculogenic, angiogenic and vascular repair properties (Abd El Aziz et al., 2015; Yu et al., 2016; Chilla et al., 2018; Kong et al., 2018). In vascular disease states and in advancing age, circulating EPC number and function are lower (Fadini et al., 2006; Thijssen et al., 2006; Xia et al., 2012;

Liao et al., 2014). It is reported that these cells are independent predictors of endothelial function (Sibal et al., 2009; Bruyndonckx et al., 2014), and may also be predictors of cardiovascular mortality (Rigato et al., 2016).

Exercise has been shown to improve endothelial function (Black et al., 2009), which is likely due to the regular elevations in shear stress (Tinken et al., 2010) that occurs due to elevated cardiac output and metabolic demand of the working muscle. Recently, acute bouts of exercise have been shown to mobilize EPCs from bone marrow and into the circulation (Van Craenenbroeck et al., 2008; Ross et al., 2014), which may contribute to endothelial growth and repair. However, in some populations, such as older individuals (Ross et al., 2018) and heart failure patients (Van Craenenbroeck et al., 2011), the acute exercise response is impaired.

Blood flow restriction (BFR) exercise has been recently used to augment muscle hypertrophy (building muscle tissue) and strength whilst undertaking low-load resistance training (Abe et al., 2012). This is of interest to individuals who are unable to undertake higher-load training, such as injured athletes, older or diseased populations. Interestingly, BFR exercise may improve vascular function compared to non-restricted exercise (matched for workload) (Horiuchi and Okita, 2012), which may make BFR exercise an option for individuals with vascular disease who cannot undertake moderate-to-high intensity exercise. One potential mechanism is the exercise-induced elevations in key angiogenic stimuli, such as vascular endothelial growth factor (VEGF), which is elevated in low-load BFR exercise compared to low-load exercise without BFR as a control (Larkin et al., 2012; Ferguson et al., 2018). This, in addition with other hypoxic stimuli, may stimulate the mobilization and recruitment of EPCs from the bone marrow, which can then act to stimulate vascular repair in areas of endothelial damage/dysfunction. Therefore we wanted to investigate the influence of BFR exercise on EPC mobilization in young, healthy men. It was hypothesized that BFR exercise would augment the exercise-induced mobilization of EPCs.

MATERIALS AND METHODS

Ethics Statement

This study was carried out in accordance with the recommendations of Edinburgh Napier University Research and Ethics Governance Committee. The study was ethically approved by Edinburgh Napier University Research and Ethics Governance Committee. All participants gave written informed consent in accordance with the Declaration of Helsinki.

Participants

Nine healthy adult males (age 18–30 years) volunteered to take part in the study. Participants were physically active (took part in formal exercise training at least 2×per week), non-obese (BMI < 30 m·kg^2), non-smokers, and not taking any medications. Participants were told to refrain from undertaking strenuous exercise for 2 days prior to the visits to the Human Performance Laboratory. Participant characteristics are provided in **Table 1**.

Experimental Design

In a repeated measures randomized design, participants performed fasted, unilateral, low-load, knee extension (KE) exercise (dominant leg) on an isokinetic dynamometer (Cybex Humac Norm, Computer Sports Medicine Inc., United States). Two experimental trials were undertaken, a low-load KE exercise (1) with and (2) without BFR, with a minimum of 1 week apart.

Assessment of Peak Torque

One week prior to the first experimental trial, participants undertook a KE maximal torque test (1RM) on the isokinetic dynamometer after a 5 min warm up on a bicycle ergometer (75 W, 60 rpm). Participants initially performed 5 repetitions, through 90° range of motion at 60° per second concentrically at ∼75% of maximal effort, followed by a short rest period before attempting a further 5 repetitions, with participants given the instruction to produce maximal efforts. 1RM was determined as the maximal voluntary torque produced throughout a controlled and full range of motion repetition. After the maximal torque assessment, participants were fitted with the pneumatic cuff placed on the dominant thigh, and performed 5 repetitions to familiarize the participants with the BFR prior to the experimental trials.

Experimental Trials

After a minimum of 7 days following the maximal torque assessment, participants returned to the Human Performance Laboratory in a fasted state, having refrained from strenuous exercise for 48 h prior to the visit, and having refrained from caffeine and alcohol the night before the visit. Participants underwent a warm up consisting of a 5 min cycle (Monark 824E, Monark Exercise AB, Sweden) at 75 W at 60 rpm, followed by 5 warm up KE repetitions at 20% 1RM. The exercise trial consisted of 4 sets of unilateral knee extensions at 20% 1RM at a cadence of 1.5 s per contraction phase across 90° range of motion and at a speed of 60° per second (1 set of 30 repetitions, followed by 3 sets of 15 repetitions) interspersed with 30 s recovery periods, similar to previous work in this area (Drummond et al., 2008; Ferguson et al., 2018). Throughout the 4 sets, participants were fitted with a thigh occlusion cuff (Hokanson CC17 Thigh Cuff, Hokanson Inc., United States) at the most proximal end of their dominant

TABLE 1 | Participant characteristics (n = 9).

Characteristics	
Age (years)	21 ± 1
Body Mass Index (m·kg^2)	25.77 ± 1.10
Systolic Blood Pressure (mmHg)	131 ± 2
Diastolic Blood Pressure (mmHg)	78 ± 2
Knee Extensor Maximal Torque (N)	255 ± 16
30% Maximal Torque (N)	75 ± 5

Values shown are mean ± SEM.

leg, either inflated to 60% of their thigh occlusion pressure (BFR) or 5 mmHg (non BFR). Thigh occlusion pressure was identified as the highest pressure at which arterial blood flow could not be detected by a vascular Doppler (BT-200 Vascular Doppler, Bistos Co., Ltd., South Korea) on the posterior tibial artery. Occlusion pressure was maintained for the entirety of the exercise bout including inter-set rest periods. Blood samples were taken pre-, immediately post- and 30 min post-exercise by venepuncture (see section "Blood Sampling and EPC Phenotyping").

All participants undertook the exercise at the same time of day as their first experimental trial (0830-1000).

Blood Sampling and Endothelial Progenitor Cell Phenotyping

Blood was taken from participants before, immediately post- and 30 min post-exercise bout by a trained phlebotomist using a 21-guage needle (BD Luer-Lok^{TM}, BD Biosciences, United Kingdom). Peripheral blood from the antecubital vein was drawn into 2 × 6 mL vacutainers spray-coated with EDTA anti-coagulant (BD Biosciences, United Kingdom), with the first 3 mL discarded to avoid contamination of circulating endothelial cells produced with the initial venepuncture. Differential leukocyte counts were determined using semi-automated hematology analyser (XS 1000i, Sysmex, United Kingdom).

For flow cytometric quantification of EPCs, briefly, 200 μL of whole blood was incubated with 5 μL of anti-CD34 FITC, 5 μL anti-CD45 BV510, and 10 μL anti-VEGFR2 PE (all BD Biosciences, United Kingdom) for 30 min away from light, followed by the addition of 2 mL Lysis (BD Pharm Lyse^{TM}, BD Biosciences, United Kingdom) prior to flow cytometric analysis. EPCs were quantified using a BD FACS Celesta (BD Biosciences, United Kingdom) flow cytometer, equipped with a Violet laser (405 nm), Blue laser (488 nm) and a Yellow-Green laser (561 nm). Compensation was performed prior to the study to correct for any spectral overlap, and controls (fluorescence minus 1) were used for each participants' visit. Circulating EPC data was obtained using BD FACS Diva (BD Biosciences, United Kingdom). Firstly, $CD45^+$ PBMCs were gated (**Figure 1A**), followed by identification of SSC-low and $CD34^+$ events (**Figure 1B**), subsequent low expression of CD45 (CD45dim; **Figure 1C**) and $VEGFR2^+$ events (**Figure 1D**) were identified. A minimum of 250,000 $CD45^+$ PBMC events were collected per sample. Circulating concentrations of progenitor cells were obtained using a dual platform method, by multiplying the percentage values obtained from the flow cytometer by the corresponding leukocyte count as obtained from hematology analysis.

Changes in blood volume was accounted for by using known measures of hematocrit and hemoglobin obtained from automated hematology analysis (Sysmex, XS 1000i, United Kingdom) (Dill and Costill, 1974).

Statistical Analysis

All data are presented as mean ± SEM unless otherwise stated. Two-way analyses of variance (ANOVA) with repeated measures were performed to investigate main effects of the exercise bout on circulating progenitor cells, and interaction of time (pre-, post-, 30 min post-exercise) × trial (BFR vs. non-BFR). When significant differences were detected, Bonferroni *post hoc* tests were performed to determine location of the effect (pre, post- 1 h post-exercise). Effect sizes are presented as Pearson's r coefficient for ANOVA analyses, and Cohen's d for paired analyses. Data was analyzed using GraphPad Prism 8 for Windows (GraphPad Software Inc., United States). Significance alpha was set at $p < 0.05$.

RESULTS

Unilateral Knee Extension Exercise Performance

There was no difference in torque produced during either BFR or non-BFR trial (75.89 ± 4.86N vs. 76.76 ± 5.96N, $p = 0.911$), which equated to 29.77 ± 1.12% vs. 31.44 ± 1.77% of maximal torque ($p = 0.423$).

Immunological Responses

There was no main effect of the exercise bout on circulating neutrophils [$F_{(2,48)} = 0.383$, $p = 0.684$, $r = 0.09$] or monocytes [$F_{(2,48)} = 1.613$, $p = 0.210$, $r = 0.18$] in the trials, but there was a main effect of exercise (pre- to post- and 30 min post-exercise) on circulating lymphocytes [$F_{(2,48)} = 13.45$, $p < 0.001$, $r = 0.47$]. However, for all three subsets of circulating leukocytes, there was no time × trial interaction ($p > 0.05$). Leukocyte changes in response to BFR and non-BFR exercise are shown in **Table 2**.

Endothelial Progenitor Cell Responses

There was no main effect of the exercise bout on $CD34^+$ progenitor cells [$F_{(2,48)} = 0.1559$, $p = 0.856$, $r = 0.06$] or any interaction of time × trial [$F_{(2,48)} = 0.2015$, $p = 0.818$, $r = 0.06$]. There was a main effect of time on $CD34^+VEGFR2^+$ EPCs [$F_{(2,48)} = 4.175$, $p = 0.021$, $r = 0.28$]. However, as with $CD34^+$ progenitors, there was no time × trial interaction [$F_{(2,48)} = 1.199$, $p = 0.310$, $r = 0.16$]. Likewise, there was a significant main effect of the exercise bout on $CD34^+CD45^{dim}VEGFR2^+$ EPCs [$F_{(2,48)} = 3.115$, $p = 0.049$, $r = 0.25$], but no significant time × trial interaction was observed [$F_{(2,48)} = 0.702$, $p = 0.501$, $r = 0.12$].

$CD34^+VEGFR2^+$ cells increased significantly from 269 ± 42 cells mL^{-1} at rest to 573 ± 90 cells mL^{-1} ($p = 0.008$, $d = 1.37$) immediately post-non-BFR exercise, with a non-significant increase of 269 ± 42 cells mL^{-1} to 373 ± 33 cells mL^{-1} in the BFR trial ($p = 0.352$, $d = 0.87$). $CD34^+VEGFR2^+$ EPCs were still significantly elevated 30 min post-exercise compared to pre-exercise levels, in the non-BFR trial only (269 ± 42 cells mL^{-1} to 564 ± 128 cells mL^{-1}, $p = 0.010$, $d = 0.98$). $CD34^+CD45^{dim}VEGFR2^+$ EPCs only significantly increased from pre- to 30 min post-exercise in the non-BFR trial (129 ± 21 cells mL^{-1} to 313 ± 103 cells mL^{-1}, $p = 0.010$, $d = 1.23$), with no such statistical differences in the BFR trial (116 ± 19 cells mL^{-1} to 177 ± 35 cells mL^{-1}, $p = 0.010$, $d = 0.68$) (**Figure 2**).

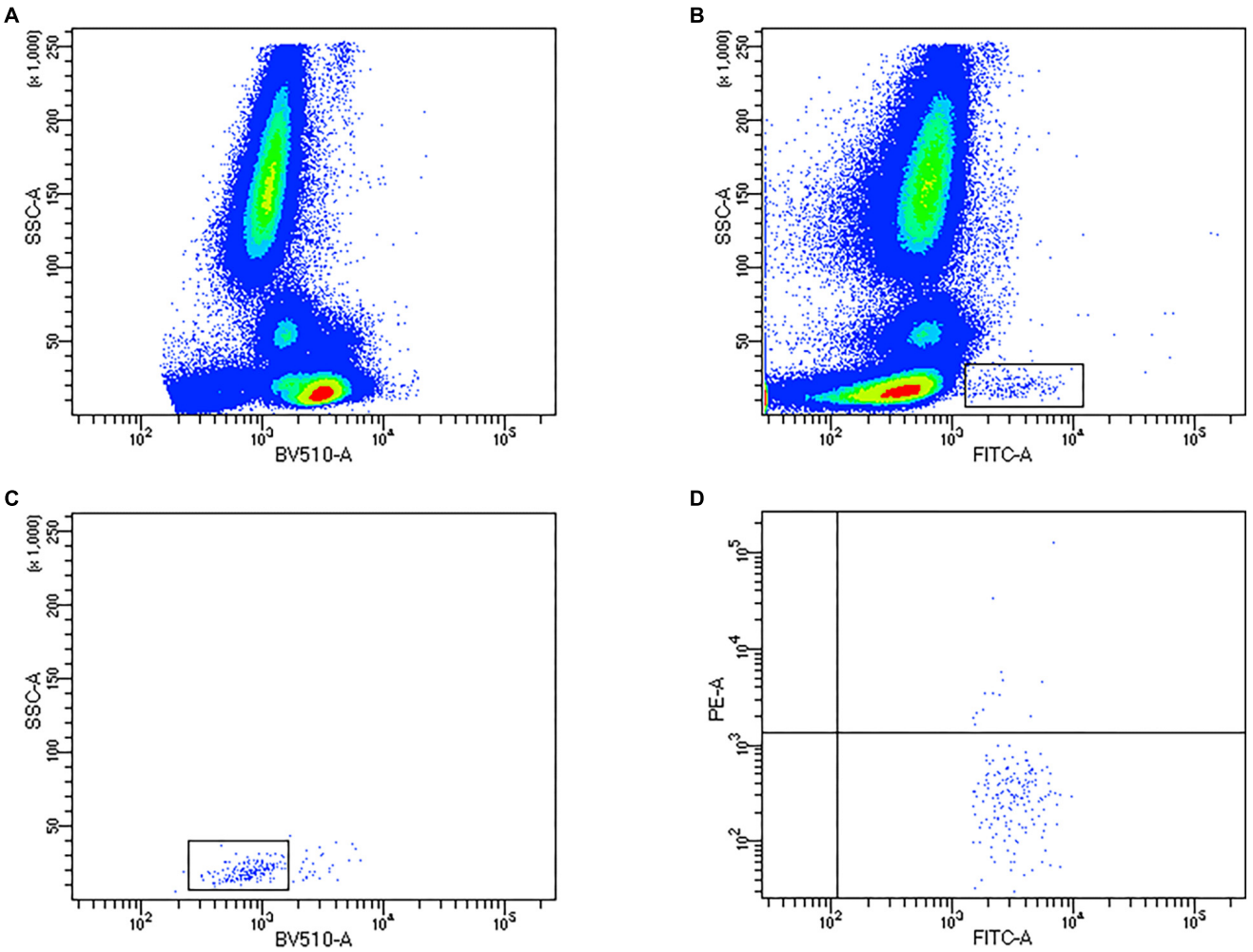

FIGURE 1 | Representative flow cytometry density and dot plots to quantify endothelial progenitor cells (EPCs). **(A)** Identification of $CD45^+$ PBMCs, **(B)** $CD34^+$ gating, **(C)** $CD45^{dim}$ expression on $CD34^+$ progenitors, **(D)** co-expression of VEGFR2.

TABLE 2 | Circulating leukocyte changes in response to blood flow restricted (BFR) and non- Restricted (non-BFR) Exercise (n = 9).

		Pre	Immediately Post-	30 min Post-	Main effect of exercise	Time x Trial Interaction
Neutrophils (cells × $10^9 \cdot L^{-1}$)	BFR	3.95 ± 0.44	4.47 ± 0.62	4.37 ± 0.59	$F_{(2,48)}$ = 0.38	$F_{(2,48)}$ = 0.13
	Non-BFR	3.38 ± 0.51	3.46 ± 0.54	3.93 ± 0.63	3, p = 0.684	7, p = 0.872
Monocytes (cells × $10^9 \cdot L^{-1}$)	BFR	0.56 ± 0.06	0.69 ± 0.08	0.56 ± 0.04	$F_{(2,48)}$ = 1.61	$F_{(2,48)}$ = 0.51
	Non-BFR	0.53 ± 0.04	0.57 ± 0.05	0.53 ± 0.05	3, p = 0.210	5, p = 0.601
Lymphocytes (cells × $10^9 \cdot L^{-1}$)	BFR	1.83 ± 0.18	2.31 ± 0.15	1.54 ± 0.12	$F_{(2,48)}$ = 13.45	$F_{(2,48)}$ = 0.98
	Non-BFR	1.95 ± 0.09	2.31 ± 0.15	1.51 ± 0.05	0, p < 0.001*	1, p = 0.382

*Values shown are mean ± SEM, *p < 0.001.*

DISCUSSION

This is the first study to investigate the effect of an acute bout of BFR exercise on circulating progenitor cells. Our main finding of the study was that BFR exercise mitigated the increase in circulating $CD34^+VEGFR2^+$ and $CD34^+CD45^{dim}VEGFR2^+$ EPCs shown in the non-BFR exercise trial. There was no statistical significant time × trial interaction, we found that only the non-BFR exercise resulted in a statistical significant increase in both $CD34^+VEGFR2^+$ and $CD34^+CD45^{dim}VEGFR2^+$ cells in the circulation, both of which resulted in a large effect (Cohen's d > 0.8). We did not observe any changes in either trial in total $CD34^+CD45^{dim}$ progenitor cells, suggestive of a specific exercise responsiveness of EPCs. We hypothesized that the BFR trial would augment the circulating EPC response to exercise, potentially due to elevation in local and systemic hypoxic and

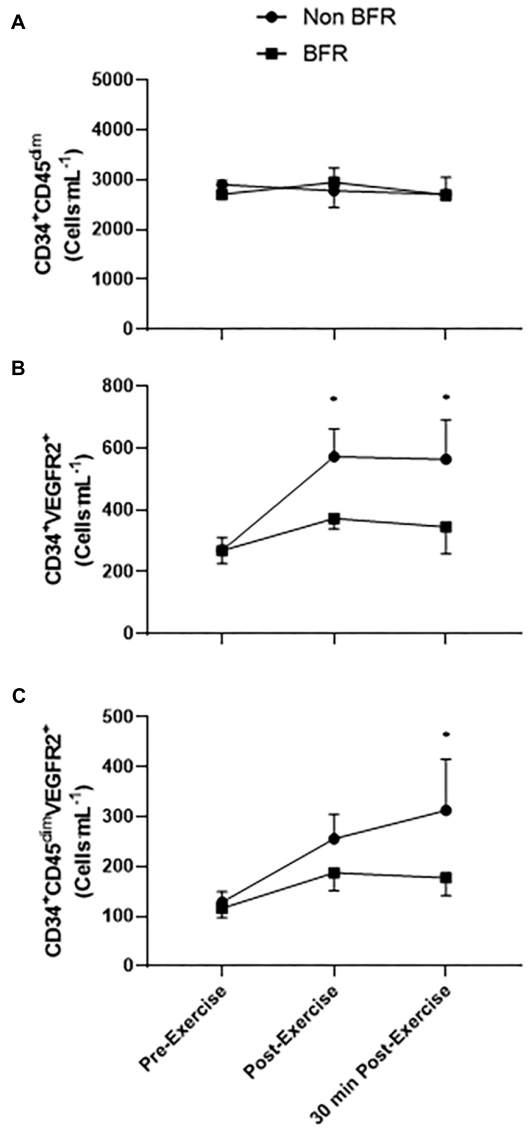

FIGURE 2 | Circulating $CD34^{+}CD45^{dim}$, $CD34^{+}VEGFR2^{+}$ EPCs, and $CD34^{+}CD45^{dim}VEGFR2^{+}$ EPCs in response to blood flow restricted (BFR) and non-restricted (non-BFR) exercise ($n = 9$). Values shown are mean ± SEM, $^{*}p < 0.05$ vs. pre-exercise non-BFR only.

angiogenic stimuli that have been shown with acute bouts of BFR exercise (Larkin et al., 2012; Ferguson et al., 2018).

Our previous work and others have shown that exercise can stimulate the mobilization of EPCs from the bone marrow of healthy young and older adults (Van Craenenbroeck et al., 2008; Ross et al., 2014, 2018). These increases in EPCs are observed concomitantly with elevations in plasma VEGF levels (Adams et al., 2004; Möbius-Winkler et al., 2009; Ross et al., 2014, 2018). Interestingly, despite elevations in VEGF mRNA, the resulting plasma VEGF concentrations did not differ between BFR and non-BFR trials in a previous BFR study (Larkin et al., 2012). Work by Ferguson et al. (2018) observed that VEGF gene expression in skeletal muscle increased in BFR exercise more so than non-BFR exercise after 2 and 4 h. It is possible that hypoxic stimulus created by the BFR exercise, may result in sustained elevation in VEGF gene expression, which may result in increased skeletal muscle VEGF protein content and subsequent elevations in VEGF released into interstitial space and plasma after 4 h. Our study focused on the initial circulating EPC response to the exercise bout, and found that BFR exercise appears to blunt the EPC mobilization immediately post-exercise, and in the short term recovery period. However, there was a moderate-to-large effect for EPC mobilization post-BFR exercise (Cohen's *d* between 0.67 and 0.87), however, this was still a lower effect than observed for non-BFR exercise. Future studies should employ further time points for analysis of EPC levels due to the possibility of any delayed VEGF release having a direct impact on EPC mobilization from the bone marrow.

Participants in the current study undertook a single leg KE exercise (of the dominant leg). Previous studies have employed BFR exercise in a bilateral exercise trial (Ferguson et al., 2018), or at a higher exercise intensity than our own (Larkin et al., 2012). We decided on a unilateral exercise trial and ∼30% of maximal torque from pilot testing for participants being able to withstand the exercise trial, however, we know that exercise intensity plays an important role in progenitor cell responses to exercise (Laufs et al., 2005), and likely that more muscle mass involved in exercise may stimulate a greater systemic response. Therefore we recommend that further studies are employed to ascertain role of exercise intensity, as well as occlusion pressure, on EPC kinetics in individuals to fully explore this area of study.

In addition to progenitor cell data, we also were able to quantify immunological response to the exercise trials. Either trial failed to stimulate significant changes in both neutrophils or monocytes. However, there was an effect of exercise on lymphocytes, with a significant redeployment of cells into the peripheral blood compartment, but there was no exercise × trial interaction. Behringer et al. (2018) observed significant elevations in absolute neutrophil count after 4 sets of BFR exercise (repetitions at 75% 1RM). Immunological responses to exercise are highly intensity-dependent (Rowbottom and Green, 2000), and therefore the difference in intensity between our 2 studies are likely to be the reason for the differences in our findings. However, our BFR trial (4 sets at ∼30% maximum torque) resulted in minimal immunological changes, and therefore may not perturb our immune system to the same extent as high intensity BFR exercise, thus making it an acceptable exercise mode for at-risk populations. However, more study is needed to investigate the influence of such bouts of exercise on specific immune cell subsets, such as T-cells, B-cells, NK-cells, and pro-inflammatory monocytes.

LIMITATIONS

Our study has several limitations which must be appreciated. Firstly, our timescale of obtaining blood samples was limited, from pre-exercise to 30 min post-exercise. We observe a delayed angiogenic gene expression in response to BFR exercise, and thus, EPC response may also be delayed, on the basis that

VEGF may stimulate exercise-induced EPC mobilization. In addition, our unilateral exercise protocol may not have been a sufficient stimulus for EPC mobilization. Despite this, we did observe a significant effect of low-intensity (∼30% maximal torque) unilateral KE exercise on EPCs in the non-restricted trial, suggestive of other factors at play other than VEGF or other angiogenic signaling proteins.

Our sample size ($n = 9$), was less than was targeted ($n > 10$ for power >95%) according to G*power calculations. However, we achieved 92% power with the $n = 9$, and as such we are confident in our analyses of the data provided, which include larger effect sizes for changes in EPCs from pre-to-post-exercise in the non-BFR trial (Cohen's d between 0.98 and 1.37) than the BFR trial (Cohen's d between 0.67 and 0.87), which failed to statistically alter the levels of EPCs in peripheral blood of the participants.

CONCLUSION

In summary, this is the first study to show that BFR exercise did not augment EPC response to exercise, and in fact blunted the EPC response to low load unilateral KE exercise in young, healthy males.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Edinburgh Napier University Research and Ethics Governance Committee. The study was ethically approved by Edinburgh Napier University Research and Ethics Governance Committee. All participants gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

MR, RM, AP, CW, GF-J designed the study. MR, RM, AP, and CW undertook the data collection. MR and RM analyzed the data. MR, GF-J wrote the manuscript. MR, RM, AP, CW, and GF-J reviewed the data and the manuscript. All authors read and approved of the manuscript.

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REVIEW
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Blood Flow Restriction Exercise: Considerations of Methodology, Application, and Safety

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The current manuscript sets out a series of guidelines for blood flow restriction exercise, focusing on the methodology, application and safety of this mode of training. With the emergence of this technique and the wide variety of applications within the literature, the aim of this review is to set out a current research informed guide to blood flow restriction training to practitioners. This covers the use of blood flow restriction to enhance muscular strength and hypertrophy via training with resistance and aerobic exercise and preventing muscle atrophy using the technique passively. The authorship team for this article was selected from the researchers focused in blood flow restriction training research with expertise in exercise science, strength and conditioning and sports medicine.

Keywords: blood flow restriction exercise, kaatsu training, occlusion training, BFR exercise, resistance training

INTRODUCTION

Blood flow restriction (BFR) is a training method partially restricting arterial inflow and fully restricting venous outflow in working musculature during exercise (Scott et al., 2015). Performing exercise with reduced blood flow achieved by restriction of the vasculature proximal to the muscle dates back to Dr. Yoshiaki Sato in Japan, where it was known as "kaatsu training," meaning "training with added pressure." Kaatsu training is now performed all over the world and is more commonly referred to as "BFR training" and achieved using a pneumatic tourniquet system (Wernbom et al., 2008; Loenneke et al., 2012d).

The technique of BFR in the muscle using a pneumatic tourniquet system involves applying an external pressure, typically using a tourniquet cuff, to the most proximal region of the upper and/or lower limbs. When the cuff is inflated, there is gradual mechanical compression of the vasculature underneath the cuff, resulting in partial restriction of arterial blood flow to structures distal to the cuff, but which more severely affects venous outflow from under the cuff that is proposed to also impede venous return. Compression of the vasculature proximal to the skeletal muscle results in inadequate oxygen supply (hypoxia) within the muscle tissue (Manini and Clark, 2009; Larkin et al., 2012). Furthermore, the diminution of venous blood flow results in blood pooling within the capillaries of the occluded limbs, often reflected by visible erythema. The level of blood pooling may be influenced by the amount of pressure applied. In addition to this, when muscular contractions are performed under conditions of BFR, there is an increase in intramuscular pressure beneath the cuff (Kacin et al., 2015), which further disturbs blood flow.

Whilst the number of research groups and studies investigating BFR have grown, so too has the number of practitioners using this mode of training (Patterson et al., 2017). This is positive, however, evidence from Patterson et al. (2017) suggests that practitioners are unclear on how to use and apply BFR in line with current research informed standards. For example, there was a wide range of pressures applied by practitioners that resulted in unintended consequences such as a large incidence of numbness following BFR. Therefore, the aim of this review is to provide a current, research informed guide to BFR from a group of world leading experts in the field. It is envisaged that this will facilitate practitioners to be more informed and clearer in deciding the reasons why they should apply BFR, how they should apply BFR as well as understanding the safety issues associated with this BFR training. For a more detailed understanding of the mechanisms of BFR exercise we refer readers to the following review articles (Wernbom et al., 2008; Pearson and Hussain, 2015).

APPLICATION OF BFR

BFR is applied during both voluntary resistance exercise (BFR-RE) and aerobic exercise (BFR-AE), and also passively without exercise (P-BFR). More recent research has examined the combination of BFR with non-traditional exercise modalities, such as whole-body vibration techniques and neuromuscular electrical stimulation.

Protocols for Enhanced Muscle Strength and Hypertrophy

In the following section, an overview of the BFR literature aiming at increasing maximal skeletal muscle strength and muscle mass will be provided. **Tables 1**, **2** provide an overview of the recommendations for the application of BFR-RE and BFR-AE, respectively.

BFR-RE

Increases in muscle hypertrophy and strength with BFR-RE are extensively documented. In recent years, a number of systematic reviews and meta-analyses have demonstrated BFR-RE to effectively increase skeletal muscle strength and/or hypertrophy in healthy young (Loenneke et al., 2012d; Slysz et al., 2016; Lixandrão et al., 2018) and older (Centner et al., 2018a; Lixandrão et al., 2018) populations, as well as load compromised populations in need of rehabilitation (Hughes et al., 2017). Various measures of muscle strength have been shown to improve in response to BFR-RE interventions, including dynamic isotonic (Burgomaster et al., 2003; Moore et al., 2004), isometric (Takarada et al., 2000a; Moore et al., 2004) and isokinetic strength (Takarada et al., 2000c, 2004 Burgomaster et al., 2003; Moore et al., 2004), as well as rate of force development/explosive strength capacity (Nielsen et al., 2017b). It is well documented that muscle hypertrophy and strength adaptations with BFR-RE are significantly greater than those achieved with low-load resistance exercise (LL-RE) alone in most (Takarada et al., 2002, 2004; Abe et al., 2005a,b,c; Yasuda et al., 2005) but not all studies (Farup et al., 2015). Such adaptations have been observed after only 1–3 weeks (Abe et al., 2005a,b, 2006; Fujita et al., 2008; Nielsen et al., 2012; Yasuda et al., 2005). These timescales for early increases in strength are mirrored in high-load resistance exercise (HL-RE) research (Blazevich et al., 2017), however, this is not typically the case for muscle mass where adaptations are not usually observed in 1–3 weeks following HL-RE (Damas et al., 2016).

Although increases in muscle size may be partly a result of the acute edema observed during and after BFR-RE (Loenneke et al., 2012c; Pearson and Hussain, 2015), improvements are still observed between 2 and 10 days post-training (Abe et al., 2005a; Fujita et al., 2008; Nielsen et al., 2012). Thus, it appears that BFR-RE allows for early addition of skeletal muscle mass; it should be noted though that this early muscle growth is likely due to the ability to use BFR-RE with a high training frequency,

TABLE 1 | Model of exercise prescription with BFR-RE.

	Guidelines
Frequency	2–3 times a week (>3 weeks) or 1–2 times per day (1–3 weeks)
Load	20–40% 1RM
Restriction time	5–10 min per exercise (reperfusion between exercises)
Type	Small and large muscle groups (arms and legs/uni or bilateral)
Sets	2–4
Cuff	5 (small), 10 or 12 (medium), 17 or 18 cm (large)
Repetitions Pressure	(75 reps) – 30 × 15 × 15 × 15, or sets to failure 40–80% AOP
Rest between sets	30–60 s
Restriction form	Continuous or intermittent
Execution speed	1–2 s (concentric and eccentric)
Execution	Until concentric failure or when planned rep scheme is completed

which is not always possible with HL-RE. For example, the lower loads used during BFR-RE to not take as long to recover from as HL-RE and thus due to these lower mechanical demands this may allow for a higher training frequency. Muscle hypertrophy with conventional training frequency (2–3 times per week) has been observed following longer training durations of 3 weeks (Ladlow et al., 2018), 5 weeks (Manimmanakorn et al., 2013), 6 weeks (Thiebaud et al., 2013), and ≥8 weeks of training (Moore et al., 2004; Libardi et al., 2015; Yasuda et al., 2016; Cook et al., 2017). BFR-RE improves muscle strength in comparison to LL-RE alone (Hughes et al., 2017) but generally show less gain in muscle strength compared to HL-RE (Loenneke et al., 2012d; Hughes et al., 2017; Lixandrão et al., 2018). Recent meta-analysis of Lixandrão et al. (2018) showed superior muscle strength gains for HL-RE as compared with BFR-RE, even when adjusting for potential moderators [e.g., test specificity (dynamic or isometric), cuff width, absolute occlusion pressure and occlusion pressure prescription method]. On the other hand, the same meta-analysis showed that BFR-RE induces comparable increases in muscle mass when compared to HL-RE, regardless of cuff width, absolute occlusion pressure and occlusion pressure prescription method. Thus, we suggest that although the muscle strength gains observed in BFR-RE are lower compared to HL-RE, the BFR is more effective than LL-RE alone and can be used when HL-RE is not advisable (e.g., post-operative rehabilitation, cardiac rehabilitation, inflammatory diseases, and frail elderly). When considering muscle mass growth, both BFR-RE and HL-RE seem equally effective. Disuse atrophy is a frequent complication in clinical populations making BFR-RE a potential alternative to HL-RE specifically for muscle mass loss.

Determining Cuff Pressure

The amount of pressure required to cease blood flow to a limb [i.e., arterial occlusion pressure (AOP)] is related to a range of individual limb characteristics; tourniquet shape, width and length, the size of the limb or an individual's blood pressure (Loenneke et al., 2012b, 2015; Jessee et al., 2016; McEwen et al., 2018). A bigger limb will require a greater cuff pressure to fully restrict arterial blood flow, and this is true across a range of cuff widths (Loenneke et al., 2012b). Some researchers have suggested that the pressure could be set relative to the individual, cuff width, and cuff material by setting the pressure relative to the arterial occlusion pressure of the cuff that will be utilized during exercise (% AOP; Patterson et al., 2017; McEwen et al., 2018). This can be done by inflating the cuff being used during exercise up to the point where blood flow ceases (100% AOP) and using a percentage of that pressure (e.g., 40–80% of AOP) during exercise. Although some have applied pressures relative to brachial systolic blood pressure (SbP) (traditional blood pressure; Brandner et al., 2015), this may not provide a consistent reduction in blood flow unless the cuff used for traditional blood pressure is the same cuff used during exercise (Loenneke et al., 2012b). How well traditional blood pressure in the arm applies to a leg (larger limb) is also something to consider with this method (Loenneke et al., 2016). Additionally, SbP has been found to correlate poorly with measurements of arterial occlusion pressure (Younger et al., 2004). Despite some researchers recommending the pressure be made relative to the exercised limb, the majority of early studies applied the same absolute pressure to each individual, independent of cuff width and limb size. These pressures have ranged from absolute pressures as low as 50 mmHg (Kubota et al., 2011) to as high as 300 mmHg (Cook et al., 2007). Although the majority of studies have produced beneficial muscular adaptations with the same absolute pressures applied to each individual, it appears that greater BFR pressure can augment the cardiovascular response and often induces discomfort associated with this type of exercise (Jessee et al., 2017; Mattocks et al., 2017). It is therefore recommended to set pressure during BFR exercise based on measurement of AOP, with pressures ranging from 40 to 80% of AOP having evidence to support their efficacy.

Cuff Width

The amount of pressure required to cease blood flow to a limb (i.e., AOP) is largely determined by the width of the cuff being applied to the limb; a wider cuff requiring a lower pressure (Crenshaw et al., 1988; Loenneke et al., 2012b; Jessee et al., 2016), essentially due to the greater surface area to which pressure has been applied. This is an important point as there are a wide range of cuff widths (3–18 cm) used in the BFR literature and setting two differently sized cuffs to the same pressure may produce a completely different degree of limb BFR (Rossow et al., 2012). It is noted that applying a relative pressure of 40% AOP does not result in a 40% reduction in blood flow (Mouser et al., 2017b). Nevertheless, a recent study found that applying pressure as a % of AOP to three different sized cuffs produce a similar change in resting blood flow (Mouser et al., 2017a). This study found that a wider cuff required less absolute pressure to restrict blood flow at any given % of AOP, but that a narrow cuff inflated to a higher absolute pressure (but same % of AOP as wide cuff) had a similar reduction in blood flow. Although lower pressures can be used with a wider cuff, this does not necessarily equate to a safer stimulus but reflects each cuff size's inherent ability to apply pressure through layers of tissue within a limb (Crenshaw et al., 1988). Lastly, we acknowledge that there may be some attenuation of growth directly under where the cuff is applied (Kacin and Strazar, 2011; Ellefsen et al., 2015), although one study suggests that this attenuation of growth may be prevented if a % of AOP is applied (Laurentino et al., 2016). Therefore, it is recommended that a wide variety of cuff widths can be used if pressure is set appropriately by using AOP. It should be noted that the wider the cuff the lower overall pressure will be needed, however, the use of extremely wide cuffs may limit movement during exercise.

Cuff Material

Throughout the literature, both elastic and nylon cuffs are commonly utilized. In the lower body (Loenneke et al., 2013, 2014b), there appears to be little difference in resting arterial occlusion or repetitions to concentric failure (surrogate for blood flow) using cuffs of the same width but made of two different materials (elastic vs. nylon). In the upper body (Buckner et al., 2017), using cuffs of different material but similar size

(3 vs.5 cm), there were large differences in resting AOP that seem unlikely explained by the slight difference in cuff width. However, when the pressure was made as a % of AOP to each cuff, the repetitions to volitional failure were similar between the two different cuff materials. This provides some evidence that the reduction of blood flow during the exercise was likely similar between cuff sizes. It appears that any difference in cuff material could be corrected for by simply applying a pressure relative to the total AOP specific for each cuff. Although studies have never directly compared cuff materials over the course of a training study, there is no available evidence to suggest that one cuff material would be superior to another. Further, both elastic and nylon cuffs have been utilized in the literature and have shown beneficial muscular adaptations (Fahs et al., 2015; Kim et al., 2017). Considering these collective findings together, the material of the cuff does not appear to impact the outcomes of BFR-RE.

Exercise Load, Volume, Rest Periods, Duration, and Frequency

Exercise Load

The pressure applied during exercise may also be dictated to some degree by the relative load lifted during resistance exercise. For the majority of individuals exercising with loads corresponding to 20–40% of an individual's maximum strength level (e.g., 1-RM) will likely maximize muscle growth and strength (Lixandrao et al., 2015; Counts et al., 2016). When loads used are at the bottom end of this recommendation (e.g., ∼20% of 1-RM), a higher pressure (∼80% AOP) may be required necessary to elicit muscle growth (Lixandrao et al., 2015), however, further study is warranted to confirm this. The majority of studies have investigated the elbow flexors and knee extensors and it is unknown whether different muscle groups require different pressure recommendations. For example, it has been suggested that targeting muscle groups proximal to the cuff may require a higher applied pressure for maximal adaptation (Dankel et al., 2016). In conclusion, we suggest that exercise loads between 20 and 40% 1RM be used because this range of loads has consistently produced muscle adaptations when combined with BFR.

Volume

In the BFR-RE literature, a common and frequently used set and repetition scheme exists that involves 75 repetitions across four sets of exercises, with 30 repetitions in the first set and 15 repetitions in each subsequent set (Yasuda et al., 2006, 2010a,b, 2011a,b, 2012; Madarame et al., 2008; Rossow et al., 2012; Ozaki et al., 2013; Loenneke et al., 2016; May et al., 2017). It is also common to complete 3–5 sets to concentric failure during BFR-RE (Takarada et al., 2002; Cook et al., 2007, 2013; Loenneke et al., 2012a; Manini et al., 2012; Nielsen et al., 2012; Ogasawara et al., 2013; Fahs et al., 2015). Furthermore, repetitions to failure may not be needed in practical settings, such as post-surgery rehabilitation of clinical populations. For example, doubling this volume of load lifted does not appear to augment any adaptations (Loenneke et al., 2011b; Martín-Hernández et al., 2013), although the dose-response relationship between volume and adaptation still needs further clarity. Therefore, it is suggested 75 repetitions, across four sets (30, 15, 15, 15) is sufficient volume to lead to adaptations in most people. Working to failure is another possibility to induce adaptations but may not always be required.

Rest Periods

Inter-set rest periods used during BFR-RE are generally short and typically the restriction is maintained throughout this period. For example, Loenneke et al. (2012d) conducted a meta-analysis that demonstrated strength adaptations with both 30 and 60 s inter-set rest periods. Some acute research has used rest periods as long as 150 s (Loenneke et al., 2010), but this was not found to increase metabolic stress any more than LL-RE, and thus may not provide training benefits. However, rest periods of both 30 s (Yasuda et al., 2010a, 2015b; Loenneke et al., 2011a) and 30–60 s (Madarame et al., 2010; Patterson and Ferguson, 2010, 2011; Yasuda et al., 2015b; Loenneke et al., 2016; Ladlow et al., 2018) are common within the BFR literature, which reflects the recommendations for achieving skeletal muscle hypertrophy (Kraemer and Ratamess, 2004). On occasions it is not always required to maintain pressure during rest periods. For example, Yasuda et al. (2013) demonstrated similar muscle activation with both continuous and intermittent pressure during rest periods, but only when a high cuff pressure was applied. Overall we recommend rest periods should constitute 30–60 s, however, intermittent BFR may reduce swelling/metabolic stress compared with continuous, which could limit the stress for adaptation.

Frequency

Traditionally, it is recommended to perform resistance training 2–4 times per week to stimulate skeletal muscle hypertrophy and strength adaptations (Fleck and Kraemer, 2004; Kraemer and Ratamess, 2004). Increases in muscle hypertrophy and strength have been reported with BFR-RE twice weekly (Takarada et al., 2000b, 2002; Laurentino et al., 2008; Madarame et al., 2008), with a recent review advocating that 2–3 BFR-RE sessions per week with progressive overload is sufficient for enhanced strength and hypertrophy adaptations (Scott et al., 2015). Some BFR research has implemented training twice daily (Abe et al., 2005b; Yasuda et al., 2005, 2010b; Nielsen et al., 2012), which may be used to accelerate recovery in a clinical rehabilitation setting (Ohta et al., 2003; Ladlow et al., 2018). In conclusion, high frequency approaches (1–2 times per day) may be used for short periods of time (1–3 weeks), however, under periods of normal programming, 2–3 sessions per week are ideal.

Duration of Training Programmes

Regarding duration of BFR-RE programmes, muscle hypertrophy and strength adaptations have been observed in short time frames, such as 1–3 weeks (Abe et al., 2005b,c; Yasuda et al., 2005; Fujita et al., 2008; Nielsen et al., 2012). Most studies have examined muscle hypertrophy and strength adaptations over time frames >3 weeks duration (Burgomaster et al., 2003; Moore et al., 2004; Abe et al., 2006; Iida et al., 2011; Nielsen et al., 2012; Yasuda et al.,

2012; Martín-Hernández et al., 2013; Luebbers et al., 2014; Kang et al., 2015).

BFR-AE

BFR-AE has been systematically reviewed (including a meta-analysis) demonstrating the effectiveness of increased strength and hypertrophy in young (Slysz et al., 2016) and older populations (Centner et al., 2018a). The application of BFR-AE usually occurs during either walking (Abe et al., 2006) or cycling exercise (Abe et al., 2010a; Conceição et al., 2019). Adaptations for strength and skeletal muscle hypertrophy have been demonstrated as early as 3 weeks (Abe et al., 2006) but most effective after at least 6 weeks of training (Slysz et al., 2016). Skeletal muscle strength has been shown to increase by 7–27% (Abe et al., 2006, 2010a,b; Ozaki et al., 2011a,b; de Oliveira et al., 2016; Clarkson et al., 2017a; Conceição et al., 2019) and hypertrophy by 3–7% (Abe et al., 2006, 2010a,b; Ozaki et al., 2011a,b; Sakamaki et al., 2011; Conceição et al., 2019) following BFR-AE. Furthermore, this mode of exercise also improves functional ability in a range of tasks (Clarkson et al., 2017a), demonstrating the impact of increased strength and muscle mass from BFR-AE on activities relevant to daily living, health and wellbeing. Alongside these changes BFR-AE can also lead to significant improvements in aerobic capacity across young (Slysz et al., 2016), old (Abe et al., 2010a), and even trained individuals (Park et al., 2010) but this is not always the case. The intensities used during BFR-AE are generally low in nature (45% heart rate reserve or 40% VO_2 max; Abe et al., 2010a; Clarkson et al., 2017a; Conceição et al., 2019), and in some cases have not been standardized (Abe et al., 2006, 2010b; Clarkson et al., 2017a) or have been implemented with a wide variety of cuff widths and pressures. A smaller body of literature has examined a variation on BFR-AE, wherein the BFR is applied immediately after the aerobic effort. Adaptations reveal an exaggerated improvement in VO_2max, and the potential for greater aerobic adaptations as a result of an acute upregulation of protein signaling (Taylor et al., 2016), as has also been shown in highly trained athletes comparing BFR-AE with matched systemic hypoxia (Christiansen et al., 2018). Unlike BFR-RE there has been a lack of standardization of pressure during BFR-AE which should be a focus in the future to optimize responses and gain greater understanding of the muscle adaptations to training with BFR-AE.

Protocols for Prevention of Strength Loss and Atrophy

In the following section, an overview over the BFR literature aiming at reducing the loss of skeletal muscle strength and muscle mass will be provided.

P-BFR

Another strategy for the use of BFR involves applying the cuffs to limbs without undertaking exercise (i.e., P-BFR). Although these approaches have not received substantial research attention, available data indicates that intermittent application of P-BFR may offset muscle atrophy and strength loss during periods of bed rest or immobilization (Takarada et al., 2000b; Kubota et al., 2008, 2011). This could theoretically provide benefits for patients following orthopedic surgeries such as anterior cruciate ligament (ACL) reconstruction and total knee arthroplasty, as less muscle mass and strength need to be regained in the rehabilitation phase. P-BFR is a similar technique to that performed during ischemic preconditioning, namely periods of ischemia followed by periods of reperfusion. To date this approach has been used to attenuate the decline in muscle mass and strength following ACL surgery (Takarada et al., 2000b), during cast immobilization (Kubota et al., 2008, 2011), and in patients in intensive care (Barbalho et al., 2018). Furthermore, P-BFR has been shown to elicit enhanced local skeletal muscle oxidative capacity and cardiovascular improvements such as increased endothelial–dependent vasodilation and vascular conductance (∼14%) in as little as 7 days (Jones et al., 2014; Jeffries et al., 2018). Similar observations have also been made following intermittent exposure over 4 weeks (Kimura et al., 2007) and 8 weeks (Jones et al., 2015).

To date, P-BFR has been implemented following a standard protocol that was originally developed by Takarada et al. (2000b). This protocol consists of 5 min of restriction followed by 3 min of reperfusion applied for 3–4 sets. Researchers have so far implemented this P-BFR once or twice per day and for a duration of 1–8 weeks (Takarada et al., 2000b; Kubota et al., 2008, 2011; Jones et al., 2014, 2015; Jeffries et al., 2018).

TABLE 2 | Model of exercise prescription with BFR-AE.

	Guidelines
Frequency	2–3 times a week (>3 weeks) or 1–2 times per day (1–3 weeks)
Intensity	<50% VO_2 max or HRR
Restriction time	5–20 min per exercise
Type	Small and large muscle groups (arms and legs / uni or bilateral)
Sets Pressure	Continuous or intervals 40–80% AOP
Cuff	5 cm (small), 10 or 12 cm (medium), 17 or 18 cm (large)
Exercise mode	Cycling or walking

TABLE 3 | Model of exercise prescription with P-BFR.

	Guidelines
Frequency	1–2 times per day (duration of bed rest/immobilization)
Restriction time	5 min intervals
Type	Small and large muscle groups (arms and legs/uni or bilateral)
Sets	3–5
Cuff	5 (small), 10 or 12 (medium), 17 or 18 (large)
Rest between sets Pressure	3–5 min Uncertain – higher pressure may be needed (70–100% AOP)
Restriction form	Continuous

It should be acknowledged though that studies have not yet investigated other protocols using different durations of BFR and reperfusion or altering the ratio of time spent with the cuff inflated vs. deflated. The pressures used during P-BFR have varied from 50 mmHg (Kubota et al., 2011) to 260 mmHg in some participants (Takarada et al., 2000b). As it stands, there is no definitive pressure allocated in the literature investigating P-BFR, although it does appear that relatively high pressures may provide the most potent protective effects against disuse atrophy given the associated complete occlusion to flow (Takarada et al., 2000b; Kubota et al., 2008). Unlike BFR exercise, the use of AOP has not been prevalent in this section of the research. It is likely that the high pressures used in some research can completely occlude blood flow to and from the limb, but this is also dependent on other factors such as cuff and limb size (Loenneke et al., 2012b).

BFR With Electrical Stimulation (BFR-ES)

Recently evidence has emerged for the use of BFR-ES. To date there is very little evidence in this area. Natsume et al. (2015) demonstrated increased muscle thickness and strength over a 2 weeks period in untrained male participants following twice per day BFR-ES. Intensity appears to have a dose-response relationship with muscular adaptation, with significant strength gains observed when a maximally tolerable stimulation intensity is used (Slysz and Burr, 2018), and positive associations between training intensity and increased in strength (r = 0.8) as well as cross-sectional area of both fast (r = 0.9) and slow (r = 0.7) fibers (Natsume et al., 2018). Furthermore, 6 weeks of unilateral low-intensity BFR-ES increased the CSA of the extensor carpi radialis longus by 17% more than ES alone in the contralateral arm of spinal cord injury patients (Gorgey et al., 2016). Alongside this, these patients also demonstrated improved vascular function, as evidenced by an increase in flow mediated dilatation (FMD). While BFR-ES is an interesting new avenue in this field, more research is needed before evidence-based recommendations for practitioners can be made.

SAFETY

The following sections will cover the safety aspects that need to be considered when implementing BFR.

Cardiovascular Response to BFR-RE

During exercise the rise of oxygen demand in the active skeletal muscles is matched by both central and peripheral vascular responses. Heart rate (HR) and stroke volume (SV) determine the total cardiac output (CO) that is distributed by vascular resistance (Hogan, 2009). Mechanisms regulating blood flow (BF) involve the central nervous system (i.e., modulation of sympathetic tone) and peripheral feedback arising from regional (i.e., venules and arterioles) and local mechanisms (i.e., the capillary beds) (Murrant and Sarelius, 2015). Local control of vasomotor tone is dependent upon metabolic, mechanical and endothelial factors. The integrated responses of increased metabolic stress, external compression of the arterial wall and shear stress of the endothelium limit autonomic sympathetic control of vasomotor tone eventually leading to a balanced level of vasodilation within active muscle that provides adequate distribution of CO (Saltin et al., 1998), and these factors are known to be affected by BFR-RE (Mouser et al., 2017a; Credeur et al., 2010). The uniqueness of BFR-RE arises from the externally applied pressure compressing blood vessels and the surrounding soft tissue that could mediate an altered cardiovascular response. Henceforth, evidences of the main central and peripheral short- and long-term vascular adaptations are presented.

Central Vascular Response to BFR-RE

The effect of BFR-RE on the central cardiovascular response is dependent upon the level of BFR (Rossow et al., 2012), mode of exercise (i.e., BFR-RE vs. BFR-AE) (Staunton et al., 2015) and mode of application (i.e., continuous vs. intermittent BFR) (Brandner et al., 2015; Neto et al., 2016). BFR acutely affects central hemodynamic parameters when it is combined with RT (Takano et al., 2005; Rossow et al., 2011, 2012; Fahs et al., 2012; Vieira et al., 2013; Downs et al., 2014; Brandner et al., 2015; Staunton et al., 2015; Neto et al., 2016; Poton and Polito, 2016; Libardi et al., 2017; May et al., 2017), AE (Renzi et al., 2010; Kumagai et al., 2012; Sugawara et al., 2015; Staunton et al., 2015; May et al., 2017) or even in the absence of exercise (Iida et al., 2007). Whilst there is an increase in the central cardiovascular response during exercise, this returns to baseline acutely (5–10 min) post-exercise cessation.

The studies that have maintained pressure during rest intervals (continuous BFR) have generally found the externally applied pressure to increase HR, SbP, diastolic blood pressure (DbP) or double product (HR × SbP) compared with the same exercise in free flow conditions (Takano et al., 2005; Renzi et al., 2010; Kumagai et al., 2012; Vieira et al., 2013; Poton and Polito, 2015; Sugawara et al., 2015; May et al., 2017). Recent works have reported contrary evidence, potentially due to the fact that occlusion pressure was set relative to AOP (Neto et al., 2016; Libardi et al., 2017). Cardiac output seems not to be affected by BFR during exercise, as BFR groups proportionally increased HR and decreased SV compared to non-BFR groups (Takano et al., 2005; Renzi et al., 2010; Sugawara et al., 2015). The removal of the BFR cuff during rest intervals (intermittent BFR) appear to mitigate cardiovascular differences between BFR and non-BFR exercise (Neto et al., 2016). Studies removing the cuff between sets or between exercises have found no further variations in HR, (Rossow et al., 2011; Fahs et al., 2012; Downs et al., 2014; Neto et al., 2016), SbP or DbP (Rossow et al., 2011; Neto et al., 2016), CO or SV (Rossow et al., 2011; Downs et al., 2014) in the BFR group compared with the non-BFR group.

Changes in central hemodynamic response are lower following BFR-RE as compared to HL-RE (Rossow et al., 2011; Fahs et al., 2012; Downs et al., 2014; Brandner et al., 2015; Poton and Polito, 2015; Libardi et al., 2017), especially if the BFR stimulus is combined with AE (May et al., 2017). However, there is evidence that alterations to the peripheral flow BFR during light walking augments both peripheral (11%) and aortic (43%) systolic pressure compared to similar exercise without occlusion. Interestingly this effect appears to be centrally mediated as BFR exerts influence only on the outgoing, but not reflected, pressure waves (Sugawara et al., 2015). Of note, pressure handling

affects the cardiovascular response to BFR-RE. Higher relative restrictive pressures induce higher cardiovascular responses to BFR-RE (Rossow et al., 2012) and may increase the potential risk associated with BFR-RE. Additionally, if pressure cuffs are not removed during rest intervals, BFR-RE could maintain blood pressure elevated as compared to HL-RE (Downs et al., 2014). On the other hand, BFR-RE results in greater post-exercise hypotension than HL-RE (Domingos and Polito, 2018).

Peripheral Vascular Response to BFR-RE

BFR exercise has been shown to affect arterial compliance and endothelial function. Vascular compliance has most frequently been tested following BFR-RE (Credeur et al., 2010; Clark et al., 2011; Fahs et al., 2012; Hunt et al., 2012, 2013). In the short term, Fahs et al. (2012) found four sets of four different lower limbs exercises to affect both large and small artery compliance. BFR-RE increased large artery compliance to the same extent as LL-RE and HL-RE, whereas small artery compliance was more affected by HL-RE with no differences between LL-RE and BFR-RE groups. These data suggest a transient improvement of endothelial function following BFR-RE. However, this acute response does not seem to preclude long-term adaptations of vascular reactivity (Clark et al., 2011; Hunt et al., 2012, 2013) which may in contrast be negatively affected by chronic low intensity BFR-RE (Credeur et al., 2010). Other forms of application of the BFR stimulus have received less attention in the literature. BFR-AE has acutely shown to impair flow mediated dilatation (FMD) (Renzi et al., 2010), whilst others have reported BFR-AE to increase FMD in the long term (Iida et al., 2011).

Systemic vascular resistance (SVR) falls in muscle exercise due to vasodilation. The threat of the systemic pressure not meeting new regulatory set-point during exercise is compensated by an increased CO and sympathetic vasomotor tone. A mismatch between CO, sympathetic control of vasomotor system and local mechanisms of active hyperemia could result in hypotensive syncope (Hogan, 2009). Syncope episodes have not frequently been reported in BFR-RE literature (Nakajima et al., 2006), but they seem to be more frequent among practitioners and clinical settings where the threat is greater in any case (Patterson and Brandner, 2017). The application of BFR in the absence of any other stimulus increases SVR with a concomitant decrease of CO (Iida et al., 2007). SVR has shown to increase or to remain unchanged following BFR-RE (Takano et al., 2005; Rossow et al., 2011; Staunton et al., 2015; Libardi et al., 2017) or BFR-AE (Renzi et al., 2010; Staunton et al., 2015) and to be reduced following exercise (Fahs et al., 2012). Although the relationship between CO and SVR does not seem to represent a cardiovascular threat in BFR exercise, a steady CO coupled with an increased SVR could drive an increase in blood pressure, and adverse individual responses may not be discarded.

Venous Thromboembolism

A thrombus is a solid mass of platelets, red blood cells, and fibrin mesh that typically forms as a response to vessel wall injury and is part of the normal healing cascade (Furie and Furie, 2008). Pooling of blood during episodes of stasis, which can happen during hospitalization or prolonged travel, can stimulate thrombus formation. A thrombus large enough to block blood flow, especially if located in the smaller vessels, can result in local tissue ischemia and subsequent tissue death. If dislodged it is termed an embolus and can result in a pulmonary embolism (PE) which can be life-threatening (Heit, 2015). Collectively, deep vein thrombus (DVT) and PE are termed venous thromboembolism (VTE).

Incidence rates of VTE have been estimated at 10 million cases annually (Raskob et al., 2014). Western Europe, North America, Australia and Southern Latin American yield consistent VTE results ranging from 0.75 to 2.69 per 1000 individuals per year. The incidence increases with age, 2–7 per 1000 in individuals >70 years old. The incidence is lower in Chinese and Korean ethnicity, however, the aging population may factor into an increasing VTE burden (Raskob et al., 2014). Several VTE risk factors have been identified and include medical conditions such as major orthopedic surgery, major general surgery, lower extremity paralysis due to spinal cord injury, pelvic, hip or long bone fractures, poly-trauma and cancer (Anderson and Spencer, 2003; Cionac Florescu et al., 2013). Additional risk factors include a history of prior VTE, obesity, immobility, oral contraceptives, family history of VTE, physical inactivity and genetic conditions that affect blood clotting (Anderson and Spencer, 2003). Pregnancy carries an elevated risk both in the perinatal and post-natal periods (Heit et al., 2005). It is much more common to develop a DVT in the lower extremities compared to the upper extremities, with approximately 10% of DVT formations being found in the upper extremities (Kucher, 2011). Finally, the insertion of central catheters during medical procedures make up the largest risk factor in upper extremities thrombus formations (Grant et al., 2012).

BFR-RE and Venous Thromboembolism: Acute Measures

There is an inherent concern in the formation of a DVT due to the external compression on vasculature via an occlusive cuff during BFR-RE. Many of the published BFR-RE trials do not directly measure for VTE formation or use diagnostic imaging. However, the totality of the literature reveals minimal adverse events pertaining to VTE and clinically reported events have not been reported.

Most studies that have assessed for VTE after the application of BFR-RE have used direct blood markers for coagulation. Acute studies have not demonstrated a significant increase in blood coagulations via D-dimer and values, one of the most utilized clinical tests to rule out the presence of a DVT, after BFR-RE exercise (Nakajima et al., 2007; Fry et al., 2010; Madarame et al., 2010; Clark et al., 2011). Madarame et al. (2013) included prothrombin fragment (PTF) and thrombin-antithrombin III complex (TAT) testing to assess for increased thrombin generation immediately after training and found no significant increase. Additionally, C-reactive protein (CRP), a protein that has been linked to clot formation, was also assessed in one study and was not significantly elevated (Clark et al., 2011). Subjects performing BFR-RE at simulated

elevation (8,000 ft) and at 6 degrees head down positioning did not demonstrate a significant rise in D-dimer, fibrin degradation product (FDP) or plasminogen activator inhibitor (PAI) (Nakajima et al., 2007). Only one study has assessed blood coagulation markers in a clinical population (Madarame et al., 2013). Nine subjects (7 men and 2 women) with a confirmed history of ischemic heart disease performed bilateral lower extremity knee extensions at 20% 1-RM with or without BFR. D-dimer, FDP and CRP were assessed before, immediately after and again 1 h after both exercise conditions. D-dimer and CRP was significantly elevated in both BFR-RE and free flow conditions, however, the values remained within a clinically normal range. Once adjusted for plasma volume (PV) the changes in each group's values were no longer statistically elevated. CRP demonstrated the same non-clinically significant rise and after PV adjustment was not significant. FDP was not statistically elevated in either group.

Most of the acute studies have been performed on healthy populations [4 healthy (Nakajima et al., 2007; Fry et al., 2010; Madarame et al., 2010; Clark et al., 2011) vs. 1 clinical (Madarame et al., 2013)]. Furthermore, sex characteristics have trended toward male vs. female subjects (38 males and 4 females), and all have been performed on the lower extremities only (Nakajima et al., 2007; Fry et al., 2010; Madarame et al., 2010, 2013; Clark et al., 2011). All studies except for one, which compared BFR-RE at 30% 1RM to an 80% 1RM free flow control group, utilized LL-RE in both BFR-RE and free flow conditions (Clark et al., 2011). Standard pressures between 150 and 200 mmHg were used in all studies (Nakajima et al., 2007; Fry et al., 2010; Madarame et al., 2010, 2013) except for one study that used a pressure equal to 130% SBP (Clark et al., 2011). Future acute studies that focus on relative pressures, the upper extremities, clinical populations and female subjects are warranted.

BFR-RE and Venous Thromboembolism: Chronic Measures

Most applications of BFR-RE in the clinical and research settings are done in a chronic manner over weeks and even months. Several papers have addressed VTE concerns in a chronic model. After 4 weeks of bilateral lower extremities exercise at 30% 1RM no changes in D-dimer, fibrinogen or CRP were noted (Clark et al., 2011). Similarly, 2 days a week for 12 weeks of BFR-RE at 20–30% 1RM did not significantly increase FDP, D-dimer or creatine kinase (CK) values in elderly subjects (ages 61–84 years; Yasuda et al., 2015a). The same authors found after 12 weeks of bilateral elbow extension and elbow flexion elastic band exercises no significant increase in D-dimer, FDP, or CK levels (Yasuda et al., 2015b). Chronic BFR-RE after knee surgery, 12 sessions over an average of 6 weeks, revealed no signs of thrombus formation as measured by duplex ultrasound scans (Tennent et al., 2017). A large epidemiologic questionnaire in Japan of over 12,000 subjects reported the incidence rate of venous thrombus at 0.055% and PE at 0.008%, of note a true medical diagnosis for PE was not confirmed (Nakajima et al., 2006). The value reported for DVT incidence in this study is lower than the reported in the general population in Asia (0.2–0.26%) which assumes a very low population risk (Klatsky et al., 2000).

The total time frame for chronic studies measuring VTE potential after BFR ranges from 4 to 12 weeks over four studies. There is much less gender bias in the chronic studies with a total of 35 men and 37 women tested. One study, utilized Doppler Ultrasound to personalize cuff pressure to 80% of AOP in the lower extremities and wide cuffs (11.5 cm; Tennent et al., 2017). The additional three studies used narrower cuffs on lower extremities (3–6 cm) and two used standard absolute pressure at an average of 196 ± 18 mmHg and the other utilized 130% of SbP (Clark et al., 2011; Yasuda et al., 2015a,b).

BFR-RE and the Fibrinolytic System

Clotting in the vascular system after injury is part of the normal healing cascade and short periods of stasis can produce thrombus formation without adverse events. One mechanism to control the advancement of thrombus formation is through stimulation of the fibrinolytic system. Resistance training has demonstrated the ability to up-regulate the fibrinolytic pathway and has been demonstrated after just one exercise session and in healthy young participants and aged patients with coronary artery disease (CAD) (El-Sayed, 1993; de Jong et al., 2006). It would appear that BFR-RE stimulates the fibrinolytic system, as application of lower extremity BFR-RE increased tissue plasminogen activator (tPA, a thrombus-degrading protein in the epithelial cell) in healthy participants (Nakajima et al., 2007; Madarame et al., 2010). Additionally, the application of vascular occlusion without exercise has demonstrated a significant increase in fibrinolytic factors (Stegnar and Pentek, 1993; Nakajima et al., 2007). However, variables such as age, sex, and obesity may alter the fibronolytic response to exercise (Stegnar and Pentek, 1993).

BFR-RE and at Risk Populations for VTE

Identifiable risk factors for VTE have been established and are a combination of endogenous characteristics such as obesity and genetic factors or exogenous triggering factors such as major surgery or pregnancy (Cushman, 2007). Advancing age is a non-modifiable risk factor for VTE formation. After the fourth decade of life, rates of incidence increase rapidly from 1 per 10,000 annually up to 5–6 per 1000 annually by age 80 (Silverstein et al., 1998). Studies addressing blood coagulation factors after BFR-RE, including D-dimer, FDP and CK, in elderly subjects have not demonstrated adverse effects. Three studies have included subjects with age ranges between 61 and 85 years and comprise both lower and upper extremities BFR-RE training (Fry et al., 2010; Yasuda et al., 2015a,b). One study has addressed BFR-RE in an aged clinical population (ischemic heart disease), and did not demonstrate an increase in blood coagulation factors (Madarame et al., 2013). Other risk factor group's coagulation status after BFR-RE has not been directly studied. However, ongoing BFR-RE clinical trials in at risk populations (dialysis patients, femur fractures and joint arthroplasty) are ongoing (Takano et al., 2013; ClinicalTrials.gov, 2016; Clarkson et al., 2017b). Established clinical prediction rules to assess VTE probability in at risk subjects can be utilized prior

to the application of BFR-RE to assist clinicians and researchers in appropriate candidates (Wells et al., 2000).

BFR-RE and Reactive Oxygen Species

Oxidative stress can occur when the generation of reactive oxygen species (ROS) exceeds the ability of the antioxidant system to reduce the molecules (Garten et al., 2015). Deflation of a tourniquet cuff is associated with an increase in ROS and has been directly associated with ischemic reperfusion injuries after orthopedic surgery (Cheng et al., 2003). Additionally, resistance exercise can induce generation of ROS (Reid and Durham, 2002; Rodriguez et al., 2003; Nikolaidis et al., 2007). Moderate exposure to ROS is necessary to induce adaptive antioxidant defense mechanisms, however, chronic or high levels of exposure have been linked to disease conditions and signaling the blood coagulation system (Alfadda and Sallam, 2012; He et al., 2016).

Blood markers of oxidative stress include protein carbonyls, lipid peroxides and blood glutathione as well as antioxidants systems. The application of BFR-RE (20% 1RM) to bilateral lower extremities did not significantly raise lipid peroxide levels (Takarada et al., 2000a). When comparing BFR, in combination with LL-RE (30% of 1RM) and moderate resistance exercise (70% 1RM) only the BFR and moderate resistance groups demonstrated increases of protein carbonyls and blood glutathione (Goldfarb et al., 2008). Similarly, BFR alone increased oxidative stress but the addition of low-level exercise to BFR (30% 1RM) significantly attenuated protein carbonyls and glutathione status. Furthermore, one exercise bout or 1 week of high-frequency BFR-RE (1–2 sessions per day/3 weeks; 20–30%-1RM) does not appear to augment oxidative stress or antioxidant enzyme response (Nielsen et al., 2017a; Centner et al., 2018b). However, moderate intensity (70% 1RM) exercise with or without BFR both significantly elevated oxidative stress (Garten et al., 2015). Thus, overall the addition of BFR to LL-RE does not appear to increase oxidative stress or antioxidant defense, thus oxidative stress formation may be load, rather than BFR-dependent. Further work to understand the effect of BFR exercise on the oxidative stress responses, and thus the potential roll for this to act as a stimulus for adaptation, are required.

Muscle Damage

Traditional HL-RE can induce muscle damage, particularly in those who are not experienced with exercise (Damas et al., 2016). This damage can be documented by direct and indirect markers and is most often associated with the eccentric phase of the exercise (Nosaka and Newton, 2002). The initial damage response is thought to occur due to overstretching of the sarcomere, resulting in z-line streaming as well as eventual disruption of the cytoskeletal matrix (Proske and Morgan, 2001). Muscle damage may also lead to activation of stretch-activated calcium channels or transient receptor potential channels which can increase intracellular calcium which can lead to destruction of sarcomeric proteins via calpain activation (Allen et al., 2005; Yeung et al., 2005). Following the initial damaging bout, there is often a secondary damage caused by the inflammatory response (Pizza et al., 2002). Given these effects, damage to the muscle can be determined directly via muscle biopsy, or it can be inferred indirectly through quantifying the symptoms thought to associate with a damaged muscle (Clarkson et al., 1986). These markers include a decrease in force production, decreased range of motion, muscle soreness, edema, and by measuring circulating levels of CK and/or myoglobin.

In extreme cases, exercise can be associated with a breakdown of striated skeletal muscle tissue, termed exertional rhabdomyolysis, that can lead to secondary pain, swelling and potential end organ damage (Tietze and Borchers, 2014). Cases of exertional rhabdomyolysis are typically associated with an exercise load that greatly exceeds the fitness and normal physical exertion of the participant, but have also been associated with high thermal loads, dehydration, or the use of certain medications (Zimmerman and Shen, 2013). It has been suggested that an exaggerated risk of rhabdomyolysis might occur as a result of BFR training, wherein metabolic stress is magnified despite the use of low-loads. Indeed, there are isolated case reports of rhabdomyolysis occurring through the use of BFR-RE (Iversen and Rstad, 2010; Clark and Manini, 2016; Tabata et al., 2016), however, analysis of the incidence rate from the published literature suggests the risk remains very low (0.07–0.2%) (Thompson et al., 2018). Survey data from Japan, where Kaatsu training has been practiced by a greater number of people, suggests a, similarly, low incidence of 0.008% (Nakajima et al., 2006). Thus, while exertional rhabdomyolysis during BFR exercise is possible, evidence does not currently suggest that the risk is inflated compared to traditional exercise.

A common concern of applying BFR with or without exercise is the possibility that this stimulus may lead to or even augment muscle damage through ischemic-reperfusion injury. Although ischemia-reperfusion injury is most commonly associated with long durations of severe ischemia (Blaisdell, 2002), it is possible that the combination of short duration BFR with muscle contraction could elevate the possibility of muscle damage with this type of exercise. The exercise-induced muscle damage response to BFR has been investigated in both the upper and lower body (Loenneke et al., 2014a). Muscle soreness, an indirect marker of muscle damage, is consistently elevated above baseline in the days following LL-RE in combination with BFR (Umbel et al., 2009; Wernbom et al., 2012; Thiebaud et al., 2013; Wilson et al., 2013; Sieljacks et al., 2016; Nielsen et al., 2017a). Large decreases in maximal torque production are often observed immediately post-exercise, however, the majority of studies suggest that torque returns back to or near baseline in the following days (Umbel et al., 2009; Wernbom et al., 2012; Thiebaud et al., 2013; Loenneke et al., 2014a). Muscle edema is consistently increased immediately post-exercise, but this edema decreases over time and is often back to baseline by 24–48 h (Thiebaud et al., 2013; Farup et al., 2015). Further, the few studies which looked at changes in range of motion found no differences across time (Thiebaud et al., 2013, 2014). While some studies have reported prolonged decrements in torque and prolonged edema, these changes are not usually different from a repetition matched control without BFR (Umbel et al., 2009; Wernbom et al., 2012) indicating that these changes are a result of LL-RE,

not the application of BFR. Although CK and myoglobin are not often measured in the studies designed to assess the time course of muscle recovery, the majority of studies do not find a change in the days following exercise or training (Abe et al., 2006; Yasuda et al., 2015a; Nielsen et al., 2017a). It is noteworthy that a recent study did observe a more prolonged decrement in torque, edema, and increases in blood proteins (CK and myoglobin) following 5 sets of blood flow restricted exercise to volitional failure (Sieljacks et al., 2016). In line, applying a high frequent protocol (1–2 sessions/day) for 2 bouts of 5 consecutive exercise days interspersed by 10 days of rest recently showed an increase in CK levels during and a decline in torque production after the first 5 days of exercise (Bjornsen et al., 2018). Notably, when these protocols were followed by a second bout of exercise 10–14 days later, there were minimal changes from baseline suggesting that there may be a repeated bout effect with this type of training (Sieljacks et al., 2016; Bjornsen et al., 2018). However, two studies applying a strenuous high frequent protocol (1–2 sessions/day) for 10–15 exercise days have shown delayed (12–20 days post-intervention) muscle mechanical adaptations presumably as a result of prolonged myocellular stress (Nielsen et al., 2017a; Bjornsen et al., 2018). To our knowledge, only two studies have investigated damage directly at the fiber level and report that although there are signs of stress, there appeared to be no or only minor damage to the actual muscle (Cumming et al., 2014; Nielsen et al., 2017a).

In summary, the available evidence suggests that the application of BFR does not appear to induce a muscle damage response to LL-RE using single exercise protocols of up to 5 sets to volitional failure. We recognize that there may be individuals who are more susceptible to muscle damage than others, however, this would seem to be driven more by inherent differences in the individual than the application of BFR. Nevertheless, easing an individual into the exercise program while documenting indirect markers of muscle damage may help to better identify those who may be more susceptible to muscle damage and help the practitioner to mitigate risk not only to BFR but exercise in general. To this point, the one study that did note damage (Sieljacks et al., 2016), observed a robust attenuation with indirect markers of muscle damage in response to the next exercise bout. The majority of the studies investigating muscle damage have applied the same pressure to each individual so it is presently unknown what impact applying a relative pressure (e.g., percentage of limb occlusion) may have on this response. In addition, most studies have been designed to investigate the time course of muscle damage following a single bout of low load resistance exercise. Thus, little is known about how this time course would be impacted by additional bouts of resistance exercise within the same week, however, current evidence suggests that 1 or 3 weeks of strenuous high-frequent (1–2 sessions/week) BFR training does not induce apparent myocellular damage in recreational active individuals (Nielsen et al., 2017a). Lastly, although there is evidence that low intensity aerobic exercise in combination with BFR may be beneficial for augmenting muscular adaptations over time, it is presently unknown if there is a damage response to this mode of exercise.

CONCLUSION

The aim of this article was to give an overview of the adaptations to different modes of BFR, methods of application and the safety considerations. The authors recommend the use of BFR combined with different forms of exercise (resisted, aerobic, passively), considering the volume and intensity, as well as the amount of cuff pressure, restriction time, size and cuff material. **Tables 1–3** set out the parameters by which practitioners should use BFR based on up to date and current research in the area.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing and reading of the manuscript. All were involved in the design and agreed to the statements made by the review.

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Six Weeks of Low-Load Blood Flow Restricted and High-Load Resistance Exercise Training Produce Similar Increases in Cumulative Myofibrillar Protein Synthesis and Ribosomal Biogenesis in Healthy Males

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Purpose: High-load resistance exercise contributes to maintenance of muscle mass, muscle protein quality, and contractile function by stimulation of muscle protein synthesis (MPS), hypertrophy, and strength gains. However, high loading may not be feasible in several clinical populations. Low-load blood flow restricted resistance exercise (BFRRE) may provide an alternative approach. However, the long-term protein synthetic response to BFRRE is unknown and the myocellular adaptations to prolonged BFRRE are not well described.

Methods: To investigate this, 34 healthy young subjects were randomized to 6 weeks of low-load BFRRE, HLRE, or non-exercise control (CON). Deuterium oxide (D_2O) was orally administered throughout the intervention period. Muscle biopsies from m. vastus lateralis were collected before and after the 6-week intervention period to assess long-term myofibrillar MPS and RNA synthesis as well as muscle fiber-type-specific cross-sectional area (CSA), satellite cell content, and myonuclei content. Muscle biopsies were also collected in the immediate hours following single-bout exercise to assess signaling for muscle protein degradation. Isometric and dynamic quadriceps muscle strength was evaluated before and after the intervention.

Results: Myofibrillar MPS was higher in BFRRE (1.34%/day, $p < 0.01$) and HLRE (1.12%/day, $p < 0.05$) compared to CON (0.96%/day) with no significant differences between exercise groups. Muscle RNA synthesis was higher in BFRRE (0.65%/day, $p < 0.001$) and HLRE (0.55%/day, $p < 0.01$) compared to CON (0.38%/day) and both training groups increased RNA content, indicating ribosomal biogenesis in response to exercise.

BFRRE and HLRE both activated muscle degradation signaling. Muscle strength increased 6–10% in BFRRE ($p < 0.05$) and 13–23% in HLRE ($p < 0.01$). Dynamic muscle strength increased to a greater extent in HLRE ($p < 0.05$). No changes in type I and type II muscle fiber-type-specific CSA, satellite cell content, or myonuclei content were observed.

Conclusions: These results demonstrate that BFRRE increases long-term muscle protein turnover, ribosomal biogenesis, and muscle strength to a similar degree as HLRE. These findings emphasize the potential application of low-load BFRRE to stimulate muscle protein turnover and increase muscle function in clinical populations where high loading is untenable.

Keywords: ischemic resistance training, deuterium oxide, remodeling, myofibrillar protein synthesis, ribosomal biogenesis

INTRODUCTION

Disease and advanced ageing can negatively affect muscle mass (Lexell et al., 1988; Mancini et al., 1992; Lindle et al., 1997) and muscle protein quality (Haus et al., 2007; Gouveia et al., 2017), which can contribute to impaired muscle contractile function (Harrington et al., 1997; Lindle et al., 1997; Brocca et al., 2017). Reductions in muscle mass and function can negatively affect mobility (Visser et al., 2005) and all-cause mortality (Metter et al., 2002; Szulc et al., 2010). Muscle mass and protein quality are determined by protein turnover. In this regard, a net positive muscle protein synthesis (MPS) increases muscle mass (Damas et al., 2016), while equivalent increases in MPS and protein degradation may reflect remodeling to maintain protein quality (Miller et al., 2014; Musci et al., 2018). Stimulating protein turnover during ageing and disease may therefore be important for preserving a functional muscle mass.

A single-bout of high-load resistance exercise (HLRE) is known to stimulate acute (~24–72 h) transient increases in net MPS (Phillips et al., 1997; Miller et al., 2005). Recent studies using deuterium oxide (D_2O) tracer methodology have demonstrated that prolonged HLRE training can stimulate cumulative increases in MPS to produce muscle hypertrophy (Brook et al., 2015; Damas et al., 2016). This impact of HLRE on MPS may partially rely on the translational capacity and activity of ribosomes (West et al., 2016). Accordingly, ribosomal biogenesis is reported to be associated with increased myofibrillar MPS (Brook et al., 2017) and muscle hypertrophy (Stec et al., 2016). In addition, satellite cell-mediated addition of myonuclei to existing muscle fibers has been proposed to contribute to muscle hypertrophy (Petrella et al., 2008), although the notion on the importance of satellite cells for muscle hypertrophy has later been challenged (McCarthy et al., 2011).

Some clinical conditions (e.g., arthritis or recovery from orthopedic surgery) may impede the use of high loading to counteract loss of muscle mass and function. In this regard, it is interesting that low-load resistance exercise regimens have proven effective at stimulating MPS as well as at promoting muscle hypertrophy and strength gains (Burd et al., 2010; Mitchell et al., 2012) in young individuals. On the other hand, low load training entails a high work volume, so it is relevant to consider alternative training regimes.

Exercise with simultaneous blood flow restriction (i.e., low-load blood flow restricted resistance exercise, BFRRE) reduces the volume of low-load exercise needed to stimulate muscle growth and strength (Fahs et al., 2015; Farup et al., 2015). Accordingly, a single bout of BFRRE has been reported to stimulate MPS (Fujita et al., 2007; Fry et al., 2010) and ≤ 6 weeks of BFRRE training has been reported to produce strength gains as well as both whole-muscle and fiber hypertrophy (Nielsen et al., 2012; Farup et al., 2015; Bjornsen et al., 2018a,b). Moreover, studies by Nielsen et al. (2012) and Jakobsgaard et al. (2018) observed increases in satellite cell and myonuclei content with ≤6 weeks of BFRRE training (Nielsen et al., 2012; Jakobsgaard et al., 2018). Interestingly, contrary the preferential type II fiber hypertrophy often seen with HLRE (Aagaard et al., 2001; Campos et al., 2002; Folland and Williams, 2007), BFRRE may direct the stress and hypertrophy more toward type I fibers (Cumming et al., 2014; Bjornsen et al., 2018a).

The myocellular responses to prolonged BFRRE are not well described. Therefore, the primary aim of the current study was to conduct a randomized controlled trial to investigate the accumulated effects of 6 weeks of BFRRE or HLRE on myocellular adaptations relating to accumulated MPS, RNA synthesis, muscle hypertrophy and strength. A secondary aim was to investigate the accumulated training effects on satellite cell and myonuclei content. We hypothesized; (1) that prolonged low-load BFRRE would be equally effective as HLRE in stimulating MPS and ribosomal biogenesis, and; (2) that both training regimens would stimulate increases in muscle fiber CSA, satellite cell content, and muscle strength.

MATERIALS AND METHODS

Subjects

Thirty-four healthy, untrained male subjects were included in the study [mean (95% CI), age 23.7 (22.9, 24.6) years; height 180.0 (178.2, 181.8) cm; weight 79.0 (74.9, 83.1) kg]. Exclusion criteria were; (1) resistance training within 6 months prior to inclusion; (2) participation in moderate/high intensity exercise training (other than resistance training) more than 1 h/week 6 months prior to inclusion; (3) use of prescription medication or intake of dietary

supplements potentially affecting muscle metabolism and growth. Written informed consent was obtained from all participants prior to inclusion. The study was approved by the Central Denmark Region Committee on Health Research Ethics (1-10-72-218-16) and registered in the database clinicaltrials.gov (NCT03380663). The study conformed to the standards for human experimental trials outlined in the Declaration of Helsinki. Results on muscle mitochondrial and metabolic adaptations from the study has been previously published (Groennebaek et al., 2018).

Study Design

Subjects were randomized to 6 weeks of low-load blood flow restricted resistance exercise (BFRRE, n = 12), high-load resistance exercise (HLRE, n = 12), or non-exercise control (CON, n = 10). Subjects in CON completed all experimental procedures except exercise. The study design comprised of an acute-trial study and a long-term study (**Figure 1**).

In the acute-trial, subjects arrived at the laboratory after an overnight fast. Subjects rested for 30 min and then consumed a protein drink containing 20 g of whey protein isolate (Whey 100 Extra Pure, Bodylab, Denmark). The protein drink was provided in the acute study to be consistent with common strength training practices. Following consumption of the protein drink, subjects performed a standardized warm-up and a single exercise bout (described in detail later). Muscle biopsies were harvested immediately (0 h) and 3 h post-exercise to assess targets related to autophagy.

For the long-term study, muscle strength tests and collection of skeletal muscle biopsies were performed before and 4 days after completion of the 6-week training period. The BFRRE and HLRE subjects repeated the muscle strength tests 14 days after completing the training intervention to assess possible delayed muscle strength adaptations following BFRRE training (Nielsen et al., 2017). To assess long-term protein synthesis rates, D_2O was orally administered throughout the training period. Collection of muscle biopsies and tests of muscle strength were performed in the early morning after overnight fasting. Subjects were instructed to maintain their habitual level of physical activity during the intervention period and to refrain from strenuous physical activity and alcohol for 3 days prior to all tests.

Deuterium Oxide Administration

Oral administration of D_2O (99.8%, Sigma Aldrich, St. Louis, Missouri, USA) was based on previous studies (Scalzo et al., 2014; Miller et al., 2015; Konopka et al., 2017) and has been described in detail previously (Groennebaek et al., 2018). The first week of the 6-week intervention included an initial loading period with subjects receiving 2 × 1 ml/kg bodyweight on the first day and 1 × 1 ml/kg bodyweight on the following 6 days. For the remaining 5 weeks, the subjects received 1 × 1 ml/kg bodyweight every second day. Plasma D_2O enrichment was assessed at the end of weeks 2, 4, and 6.

Blood Flow Restriction

Standardization of blood flow restriction for subjects in the BFRRE group was achieved by prescribing cuff pressures relative to individually determined arterial occlusion pressures (AOP) as previously described (Sieljacks et al., 2017). In short, AOP was determined in a supine position by incrementally inflating a 14-cm pneumatic cuff (Delfi Medical, Vancouver, Canada) using a digital tourniquet (A.T.S 2200TS, Zimmer Surgical Inc. Ohio, USA). Pressure was increased until auscultatory pulse was no longer detectable in the posterior tibial artery using Doppler ultrasound (Dopplex-D900, Huntleigh Healthcare Ltd., UK). AOP was determined at pre and was reassessed at training bout 9. Cuff pressure was set to 50% of AOP during BFRRE. Mean [95% CI] cuff pressure was 79 [74, 84] mmHg in training bout 1–8 and 78 [76, 82] mmHg in bout 9–18.

Training Protocols

A detailed description of the training intervention has previously been published (Groennebaek et al., 2018). Exercise was practiced

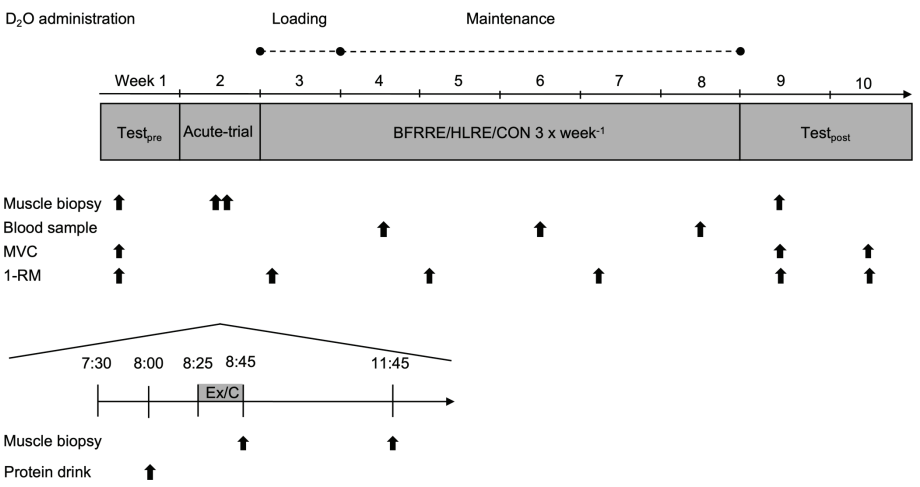

FIGURE 1 | Study overview. Pre-test was conducted 2 weeks prior to the 6-week training period. MVC maximal voluntary contraction; 1-RM one-repetition maximum; Ex, exercise; C, control.

in accordance with recommended principles (i.e., BFRRE; low load, many repetitions, short inter-set recovery and HLRE; high load, few repetitions, long inter-set recovery) (ACSM, 2009; Scott et al., 2015). In short, subjects in the two training groups underwent supervised BFRRE or HLRE training 3×/week for 6 weeks. Each training session consisted of a standardized warm-up and a main training bout. For subjects allocated to BFRRE, training bouts consisted of four sets of knee-extension exercise during continuous blood flow restriction (50% of AOP). The training load was set to 30% of 1-RM (repetition maximum) and inter-set recovery was 30 s. For subjects allocated to HLRE, training bouts consisted of knee-extension exercise in four sets of 10–12 repetitions with 3 min inter-set recovery. The training load was set to 70% of 1-RM. For both training groups, the training load was re-adjusted to a corresponding 3-RM test, two times during the 6-week intervention period (weeks 3 and 5). Furthermore, the training load for HLRE was increased if 12 repetitions could be completed for all 4 training sets and decreased if subjects were unable to perform 10 repetitions in the first training set.

Unilateral Maximal Isometric Knee Extensor Muscle Strength

Following a 5-min warm-up consisting of low intensity (~100 W) ergometer biking (Monark Ergomedic 818E, Monark, Varberg, Sweden), the subjects were seated on an isokinetic dynamometer (Humac Norm, CSMI, Stoughton, Massachusetts, USA) with restraining straps put on the torso and hips to avoid accessory movements. Subjects were positioned with 90° hip flexion and the rotational axes of the knee and the dynamometer lever arm aligned. The subjects' dominant leg was attached to the dynamometer arm ~3 cm proximal to the medial malleolus. Maximal voluntary contraction (MVC) was measured at 70° knee-flexion (0° = full extension). Subjects were instructed to initiate the contraction as "fast and forcefully as possible" and to avoid any countermovement. Each contraction lasted ~3 to 4 s and subjects were given verbal encouragement and visual feedback during the trials. A minimum of four trials were given and trials were separated by 1 min recovery time. Additional trials were provided to subjects that continued to improve. Torque recordings were sampled at 1,500 Hz and analyzed offline using a custom-made software (Labview 2011, National Instruments Corporation, Austin, Texas, USA). All trials were visually inspected and trials displaying countermovement at the onset of contraction, were excluded from the analysis. MVC was defined as the peak torque recording from the included trials. Test of MVC was conducted at pre, at 4, and 14 days post-exercise.

Dynamic Maximal Knee-Extensor Muscle Strength (3-RM)

Bilateral knee-extensor 3-RM was assessed in a knee-extension machine (Technogym Selection Line Leg Extension, Technogym SpA, Cesena, Italy) on six occasions; at pre-testing (used for familiarization), immediately before the first training bout in week 1 (used as pre-value), at the first training bout in training weeks 3 and 5 (used for adjustment of training load), at 4 days post-exercise, and at 14 days post-exercise. Warm-up consisted of 5 min of low-intensity (~100 W) ergometer biking (Monark Ergomedic 818E, Monark, Varberg, Sweden) and two warm-up sets of five repetitions with loads corresponding to 50 and 70% of estimated 1-RM. For each successful 3-RM trial the load was increased by a minimum of 2.5 kg until the subject was unable to reach full knee extension for three repetitions. A minimum of 2 min of rest was given between trials and 3-RM was generally determined within five trials. The subjects' 1-RM was estimated from their 3-RM using the following equation; $1\ RM = (3\ RM)/[1.0278 - (3 \times 0.0278)]$ (Brzycki, 1993).

Muscle Biopsy Sampling and Preparation

Before and after the 6-week intervention period, muscle biopsies (~120 mg) were collected from *v. lateralis* distally to the occlusion site under sterile conditions and local anesthesia (1% Lidocaine, Mylan Hospital, Norway) using the Bergström needle technique (Bergstrom, 1975). All biopsies were harvested at ~1 to 2 cm depth. Pre- and post-intervention biopsies were collected from the same leg (randomized for leg dominance) with ~3 cm between sampling sites (Vissing et al., 2005). Immediately following collection, biopsies were dissected free of visible fat and connective tissue. Tissue for fractional synthesis rate (FSR) analysis (~50 mg) and immunoblotting (~30 mg) were frozen in liquid nitrogen. Tissue for immunohistochemical analysis (~40 mg) was embedded in Tissue-Tek (Leica Biosystems, Nussloch, Germany) and frozen in isopentane pre-cooled in liquid nitrogen. Biopsies were stored at −80°C.

Tissue Preparation for Measurement of Myofibrillar Protein FSR

Body water enrichment and tissue alanine enrichment were determined from plasma as previously described (Robinson et al., 2011; Drake et al., 2013; Scalzo et al., 2014; Konopka et al., 2017). Approximately 25–50 mg of skeletal muscle was homogenized in an isolation buffer containing 100 mM KCl, 40 mM Tris HCl, 10 mM Tris base, 5 mM $MgCl_2$, 1 mM EDTA, and 1 mM ATP (pH 7.5), with phosphatase and protease inhibitors (HALT; ThermoScientific, Rockford, IL, USA) with a bead homogenizer (Next Advance, Inc., Averill Park, NY, USA). After homogenization, the samples were centrifuged at 800 *g* for 10 min at 4°C. The resulting pellet enriched with myofibrillar proteins was isolated and washed with 500 µl of 100% ethanol and rinsed with 500 µl of distilled water twice. The pellet was resuspended in 250 µl of 1 M NaOH and placed on a heat block for 15 min at 50°C shaking at 900 rpm. The myofibrillar protein enriched fraction was then incubated in 6 N HCl for 24 h at 120°C for protein hydrolysis. The hydrolysates were ion exchanged, dried in a vacuum, and resuspended in 1 ml of molecular biology grade H_2O. Half of the suspended sample was derivatized by a 1-h incubation of 500 µl acetonitrile, 50 µl K_2HPO_4, pH 11, and 20 µl of pentafluorobenzyl bromide. Ethyl acetate was added and the organic layer was removed, dried under nitrogen gas, and reconstituted in 600 µl ethyl acetate for analysis on an Agilent

7890A GC coupled to an Agilent 5975C MS as previously described (Robinson et al., 2011; Scalzo et al., 2014; Konopka et al., 2017). The newly synthesized fraction (*f*) of myofibrillar proteins was calculated from the enrichment of alanine bound in muscle proteins over the entire labeling period, divided by the true precursor enrichment (*p*), using the average plasma D_2O enrichment over the period of measurement with MIDA adjustment (Busch et al., 2006).

RNA Extraction and Measurement of FSR

Approximately 15–25 mg of skeletal muscle was homogenized in 800 µl of Trizol (ThermoFisher, Rockford, IL, USA) using a bead blender. The homogenate was centrifuged at 12,000 *g* for 10 min at 4°C. The resulting supernatant was removed and 160 µl of chloroform was added. The mixture was shaken vigorously then centrifuged at 12,000 *g* for 15 min at 4°C. The upper aqueous layer was isolated, mixed with 400 µl of isopropanol, and then left to incubate at room temperature for 10 min. After incubation, the mixture was centrifuged for 10 min at 4°C to pellet RNA. The RNA pellet was isolated, rinsed with 800 µl of 75% ethanol, and resuspended in 50 µl of molecular biology grade H_2O. RNA synthesis (~85% of total RNA exists as ribosomal RNA) was determined by deuterium incorporation into purine ribose of RNA as previously published (Mathis et al., 2017). The isolated RNA was hydrolyzed overnight at 37°C with nuclease S1 and potato acid phosphatase. Hydrolysates were reacted with pentafluorobenzyl hydroxylamine and acetic acid and then acetylated with acetic anhydride and 1-methylimidazole. Dichloromethane extracts were dried, resuspended in ethyl acetate, and analyzed on an Agilent 7890A GC coupled to an Agilent 5975C MS. For GC-MS analysis, we used a DB-17 column and negative chemical ionization, with helium as carrier and methane as the reagent gas. The fractional molar isotope abundances at m/z 212 (M0) and 213 (M1) of the pentafluorobenzyl triacetyl derivative of purine ribose were quantified using ChemStation software. All analyses were corrected for abundance with an unenriched pentafluorobenzyl triacetyl purine ribose derivative standard. For the precursor enrichment, the average D_2O enrichment over the period of measurement was adjusted by MIDA for ribose equilibration (Busch et al., 2006).

Immunohistochemistry

Serial transverse 10 µm cross-sections were cut from the embedded biopsy at −18°C using a cryostat (CM3050S, Leica Biosystems, Nussloch, Germany) and mounted on glass slides (Superfrost Ultra Plus, Thermo Scientific, Germany). Cross-sections were stored at −80°C until later analysis.

Muscle Fiber Cross-Sectional Area

Muscle biopsy cross-sections were placed in room temperature and allowed to thaw and dry. Sections were fixed in Histofix (Histolab, Gothenburg, Sweden) for 4 min followed by 1.5 h blocking in blocking buffer (2% BSA, 5% FBS, 2% goat serum, 0.2% Triton x-100, 0.1% sodium azide). Sections were incubated overnight at 4°C in primary antibody MHC-I (1:1,000; cat. no. A4.951, Developmental Studies Hybridoma Bank, IA, USA) for distinction of muscle fiber type I. Next, sections were incubated with Alexa-fluor 568 goat anti-mouse (1:500; cat. no A11004, Molecular Probes, Invitrogen A/S, Taastrup, Denmark) secondary antibody for 1 h followed by incubation with 488 mouse anti-Human Collagen IV (1:100; cat. no. 53-9871, Affymetrix, CA, USA) antibody for 1 h for visualization of muscle fiber border. A cover slip was applied on sections using mounting medium (Cat. no. P36930, Molecular Probes Prolong Gold anti-fade reagent, Invitrogen A/S, Taastrup, Denmark) and stored at −20°C until further analysis. Washing in three changes of 1% PBS was carried out between all steps. Antibodies were diluted in 1% BSA.

Images were captured at 10× magnification with a Leica DM2000 microscope and a Leica DFC450 Hi-resolution Color DFC camera (Leica Microsystems, Broenshoej, Denmark). Muscle fiber border and muscle fiber type were identified using semi-automatic segmentation software (Smith and Barton, 2014). Manual correction of the initial segmentation and fiber-typing was made before determination of fiber-type-specific CSA and fiber-type distribution. Mean [95% CI] number of fibers included in the analysis of muscle fiber CSA and fiber-type distribution were 215 [188, 242] for type I fibers and 255 [217, 292] for type II fibers. Total fiber area was computed as a weighted mean of type I and type II CSA.

Satellite Cells and Myonuclei

Cross-sections were initially prepared as described above. After blocking, the sections were incubated overnight at 4°C in primary antibody Pax7 (1:500; cat. no, MO15020, Neuromics, MN, USA) for visualization of SCs followed by incubation in Alexa flour 568 goat-anti-mouse secondary antibody (1:200; cat. no. A11004, Invitrogen A/S, Taastrup, Denmark) for 1.5 h. Next, sections were incubated with a mixture of primary antibodies against MHC-I (1:500; cat. no. A4.951, Developmental Studies Hybridoma Bank, IA, USA) and laminin (1:500; cat. no. Z0097, Dako Norden, Glostrup, Denmark) for 2 h for distinction of muscle fiber type I and muscle fiber border, respectively. Secondary antibodies Alexa Fluor 488 goat anti-mouse and Alexa Fluor 488 goat anti-rabbit (1:500; cat. no A11029 and A11008, Invitrogen A/S, Taastrup, Denmark) were mixed an applied to the sections for 1 h. A cover slip was applied on sections using mounting medium containing 4′,6′-diamidino-2-phenylindole (DAPI) which stains the nuclei (Cat. no. P36935, Molecular Probes Prolong Gold anti-fade reagent, Invitrogen A/S, Taastrup, Denmark). Sections were stored at −20°C until further analysis. Washing in three changes of 1% PBS was carried out between all steps. Antibodies were diluted in 1% BSA.

Images were captured at 20× magnification with a Leica DM2000 microscope and a Leica DFC450 Hi-resolution Color DFC camera. The number of SCs associated with type I or type II fibers were quantified separately by counting cells characterized by co-localization of Pax7 and DAPI inside the basal lamina of distinct muscle fibers. Satellite cells (SC) were expressed relative to the total number of type I and type II fibers (SC/fiber) and CSA (SC/mm^2). For quantification of myonuclei, we counted Pax7 negative nuclei with a geometric

center within the basal lamina (Bruusgaard et al., 2012). Number of myonuclei was expressed relative to the total number of type I and type II fibers (myonuclei/fiber) and as myonuclear domain (μm^2/myonuclei). The number of fibers analyzed for SCs and myonuclei was based on Mackey et al. (2009). For SC analysis, we counted a mean [95% CI] number of 154 [136, 172] type I fibers and 165 [144, 187] type II fibers. For myonuclei analysis, we counted 83 [77, 88] type I fibers and 89 [80, 97] type II fibers.

Immunohistochemical image analysis was conducted with the investigator blinded for subject ID and time of sample collection. Fibers situated on the edge of the cross-sections as well as fibers characterized by poor morphological integrity were excluded from analysis.

Immunoblotting

Frozen muscle tissue was freeze-dried, homogenized, separated by sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE), and electroblotted onto PVDF membranes as previously described (Rahbek et al., 2015). Primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA) and used as follows; p-FoxO3a (Ser^{253}) (conc. 1:1000, cat # 3938) p-ULK1Ser555 (1:2,000; cat. no. 5869) and LC3B (1:1,000; cat. no. 3868). P-FoxO3a was diluted in 1% BSA. The other primary antibodies were diluted in 5% BSA. After overnight incubation in primary antibodies, membranes were incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit (cat. no. 6721 ABCAM, Cambridge, UK) in a 1:5,000 solution with 1% BSA. Proteins were visualized by chemiluminescence (Thermo Scientific, Waltham, MA, USA) and quantified with an UVP imaging system (UVP, Upland, CA, USA). Arbitrary protein intensity was normalized to total amount of protein loaded in the corresponding lanes using Stain Free Technology as previously described (Gilda and Gomes, 2013; Gurtler et al., 2013).

RNA Purification and Analysis

Approximately 15–30 mg of muscle tissue was homogenized using a Precellys 24 (Bertin Technologies, France). Purification of total RNA was carried out using a QIAGEN RNeasy Mini Kit (cat. #217004, QIAGEN, Germany) according to the manufacturer's instructions. Quantification of RNA was determined by measuring the absorbance at 260 nm using a spectrophotometer (NanoDrop 1,000; Thermo Scientific, IL, USA). As ~85% of RNA exists as ribosomal RNA, total RNA (ng/mg tissue) was considered a marker of ribosomal abundance.

Statistical Analysis

Differences between groups in myofibrillar MPS and RNA synthesis were evaluated with a one-way ANOVA. Statistical analysis of muscle fiber CSA, satellite cell, myonuclei, muscle strength, and immunoblotting data were performed using a linear mixed model with group, time, and time × group interaction as the factors of interest. Model validation included test for equal standard deviations and examination of QQ plots. Associations between variables were evaluated using linear regression and Pearson's correlation. Using K-means cluster analysis, subjects in the two training groups were pooled and separated into non-responders and responders based on the magnitude of relative changes in total muscle fiber CSA as previously done by others (Stec et al., 2016). The cluster algorithm made the two clusters to minimize the sum of the squared distances to the cluster centers. Subsequently, analysis of differences between non-responders and responders were performed using a linear mixed model with cluster, time, and time × cluster interaction as the factors of interest. Alpha level was set to $p \leq 0.05$. Graphic data are presented as mean ± SD. Data in tables and text are presented as mean with 95% CI. Statistical analysis was made in Stata 15.0 (Statacorp, College Station, TX, USA) and graphical presentations were made in GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Subjects

All subjects completed the intervention. Baseline characteristics are presented in Table 1 in Groennebaek et al. (2018). Two subjects in the HLRE group completed 15 and 17 of the 18 scheduled training sessions. The remaining 20 subjects in the two training groups completed all 18 training sessions.

Training Parameters

Training parameters averaged over the training period are presented in (Groennebaek et al., 2018). When compared to HLRE, BFRRE training was characterized by a higher number of performed repetitions, but a lower training load and training volume.

Muscle Fiber-Type-Specific Cross-Sectional Area

The effect of the intervention on changes in muscle fiber CSA is described using fiber-type-specific mean CSA (**Figure 2**) and area-frequency distribution (**Supplementary Figure S1**). As shown in **Figure 2**, no changes in the CSA of type I or type II fibers were observed in either group. Moreover, area-frequency curves revealed no changes in the proportion of smaller or larger fibers in any of the groups (**Supplementary Figure S1**).

Long-Term Myofibrillar Protein Synthesis and RNA Synthesis

Average D_2O body water enrichment was stable throughout the labeling period as reported previously (Groennebaek et al., 2018). As shown in **Figure 3**, both training groups had higher myofibrillar MPS and RNA synthesis compared to CON. No differences in myofibrillar MPS or RNA synthesis were observed between BFRRE and HLRE. Myofibrillar protein FSR was correlated to RNA FSR ($R^2 = 0.42$, $p < 0.001$; **Figure 3A**). However, neither RNA FSR nor myofibrillar protein FSR were correlated to the

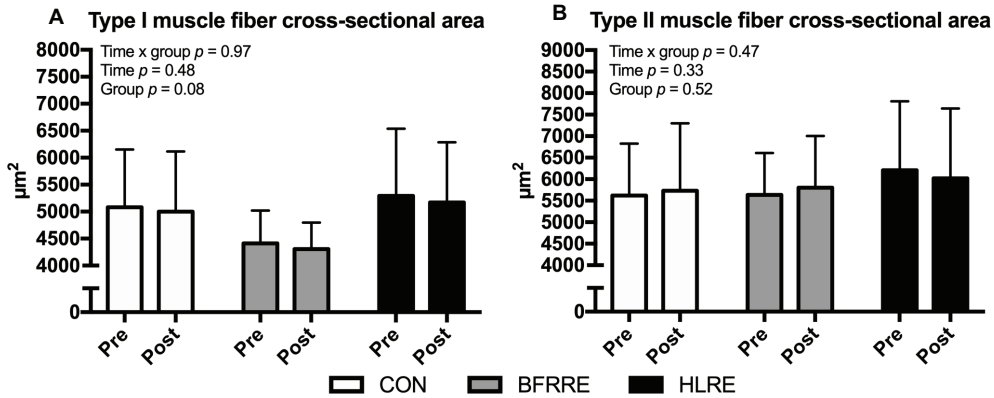

FIGURE 2 | Muscle fiber cross-sectional area of type I fibers **(A)** and type II fibers **(B)** at baseline (pre) and 4 days after cessation of training (post). Overall effects are given in the upper left corner of graphs of **(A,B)**.

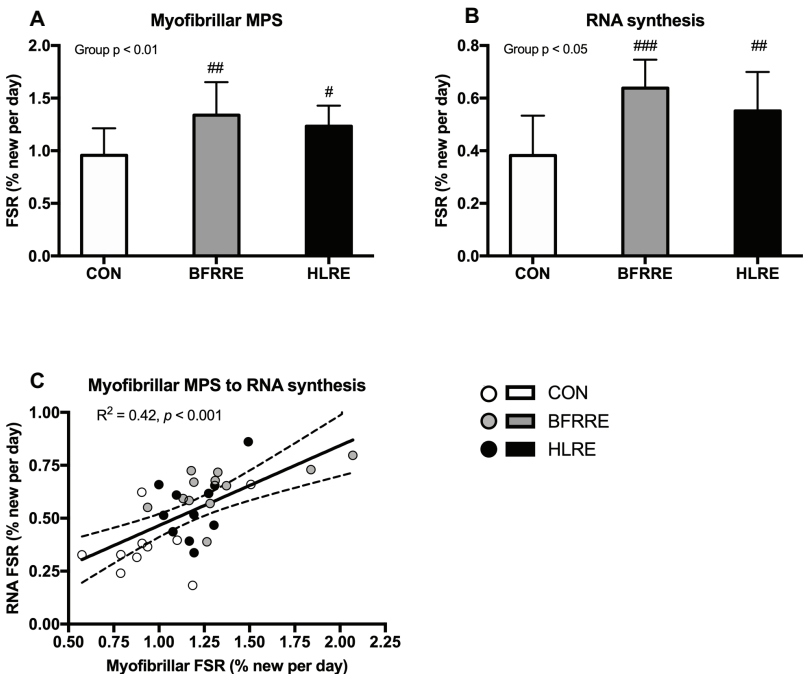

FIGURE 3 | Myofibrillar protein **(A)** and RNA **(B)** synthesis rates (%/day) during the intervention period. Correlation between myofibrillar FSR and RNA FSR **(C)**. Data are presented as mean ± SD in **(A,B)**. In **(C)**, data are presented as individual values and a linear regression line (solid) with 95% CI (dashed). Overall group effect is given in the upper left corner of graphs of **(A)** and **(B)**. *R* square and significance is given in the upper left corner of **(C)**. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ different from CON.

percentage change in total muscle fiber area ($R^2 = 0.09$, $p = 0.18$, and $R^2 = 0.09$, $p = 0.16$, respectively, data not shown).

Total RNA

Overall effects of time, group and time × group was detected for total RNA content ($p < 0.05$). As shown in **Figure 4A**, total RNA content increased from pre to post in BFRRE and HLRE. At post, total RNA content was higher in BFRRE compared to CON. No changes were observed in CON. No correlation between RNA synthesis and change in total RNA content was observed (**Figure 4B**). The change in total RNA content was not correlated to RNA synthesis (**Figure 4B**) or MPS ($R^2 = 0.004$, $p = 0.74$, data not shown).

Autophagy Signaling

BFRRE and HLRE increased phosphorylation of ULK1 at Ser^{555} immediately (0 h) following exercise (**Figure 5A**). Moreover, the ratio of LC3B2 to LC3B1 decreased after HLRE and tended to decrease 3 h after BFRRE (**Figure 5B**). In HLRE, this was mainly driven by a decreased protein expression of LC3B2

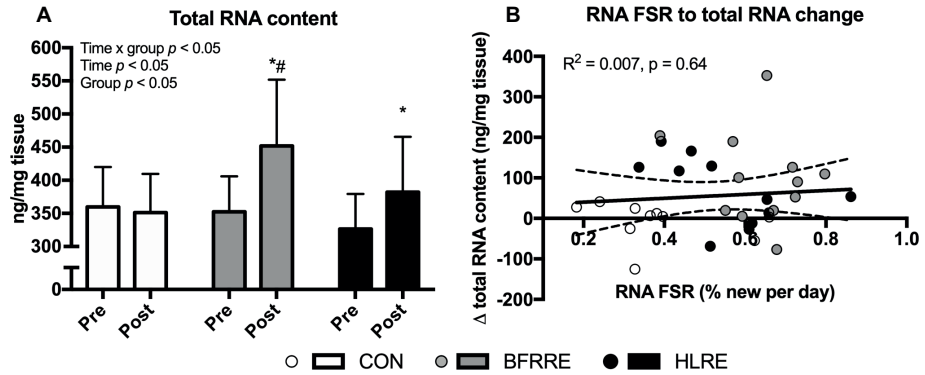

FIGURE 4 | Total RNA content at baseline (pre) and 4 days after cessation of training (post) **(A)**. Correlation between RNA synthesis and change in total RNA content. Data are presented as mean ± SD in **(A)**. In **(B)**, data are presented as individual values and a linear regression line (solid) with 95% CI (dashed). Overall effects are given in the upper left corner of graphs **(A)**. R square and significance is given in the upper left corner of **(B)**. $^{*}p < 0.05$ different from pre within group; $^{\#}p < 0.01$ different from CON.

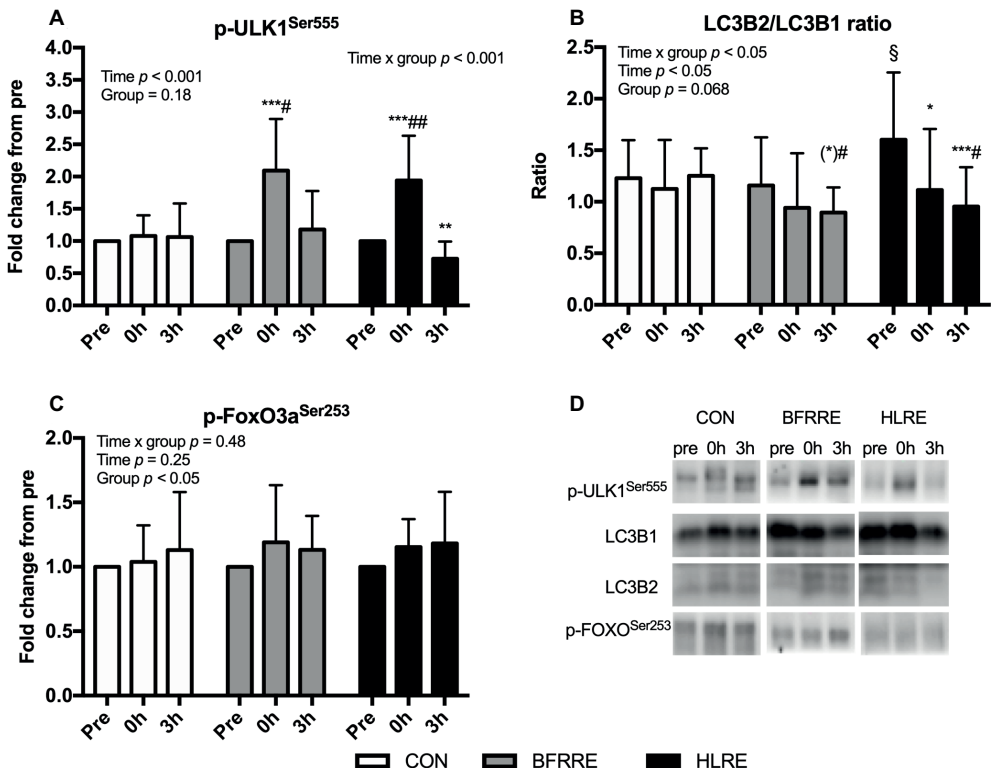

FIGURE 5 | Phosphorylation of ULK1 at Ser^{555} **(A)**, the ratio of LC3B2 to LC3B1 **(B)** protein expression, and **(C)** phosphorylation of FoxO3a at Ser^{253} immediately (0 h) and 3 h (3 h) after acute exercise. Data are presented as mean ± SD. Overall effects are given in the upper left corner of graphs. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ different from pre within group; $^{(*)}p < 0.1$ tendency toward difference from pre within group; $^{\S}p < 0.05$ different from BFRRE within time-point; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ different from CON within time-point. Representative blots are shown in **(D)**.

while the tendency in BFRRE emerged owing to non-significant up- and downregulation of LC3B1 and LC3B2 expression, respectively (data not shown). No changes in phosphorylation of FoxO3a at Ser^{253} were observed in any of the groups (**Figure 5C**). No changes were observed in CON in any of the autophagy-related targets analyzed. Representative immunoblots are shown in **Figure 5D**.

Satellite Cells

No changes in fiber-type-specific number of satellite cells were observed when satellite cells were normalized to number of fibers (**Figures 6A,B**) or fiber CSA (**Figures 6C,D**). Similarly, no changes were observed when the number of satellite cells were expressed as a percentage of the total number of nuclei [SC/(SC + myonuclei) × 100] (**Supplementary Figure S2**).

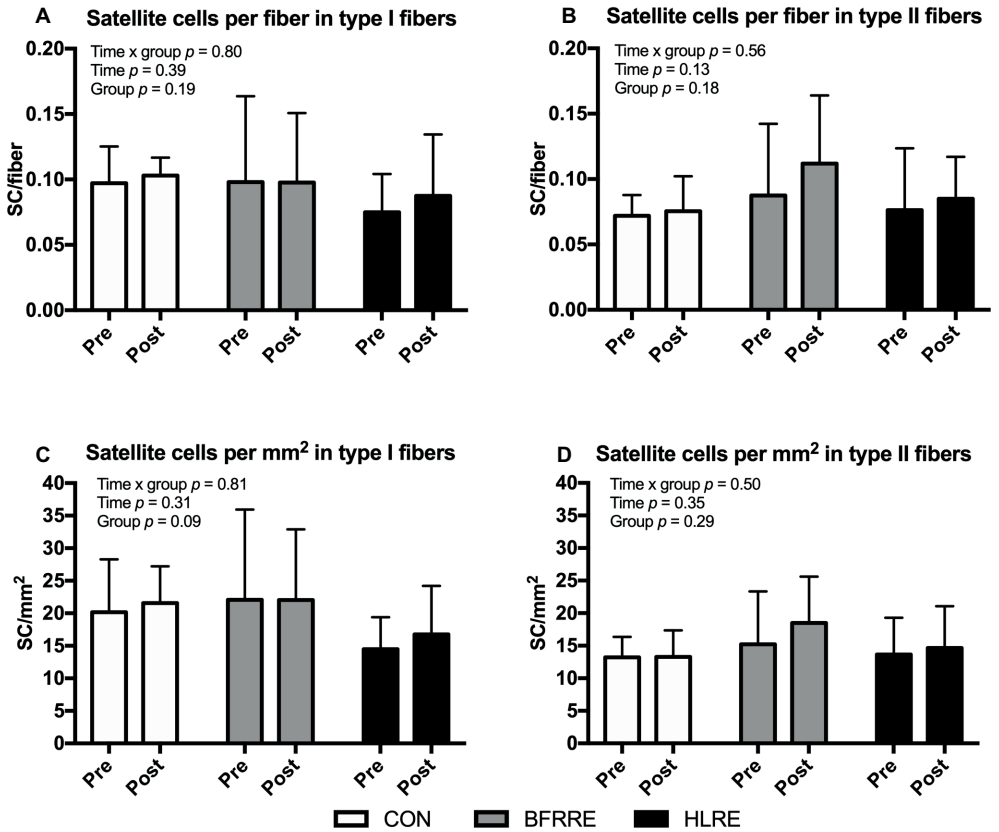

FIGURE 6 | Number of satellite cells at baseline (pre) and 4 days after cessation of training (post) expressed relative to number of fibers **(A,B)** and fiber CSA **(C,D)**. Data are presented as mean ± SD. Overall effects are given in the upper left corner of graphs.

Myonuclei

As shown in **Figure 7**, no changes in fiber-type-specific number of myonuclei per fiber or myonuclear domain were observed.

Muscle Strength

Changes in muscle strength are shown in **Figure 8**.

Isometric Muscle Strength (MVC)

An overall time × group interaction for MVC was detected ($p < 0.05$). No changes were observed in CON. HLRE increased MVC from pre to post (13.6 [4.8, 22.4]%) and from pre to 14 days after cessation of training (13.1 [3.4, 22.8]%). In BFRRE, MVC increased from pre to 14 days after cessation of training (6.2 [−0.3, 12.6]%). No between-group differences were observed.

Dynamic Muscle Strength (1-RM)

An overall time × group interaction for 1-RM was detected ($p < 0.05$). No changes were observed in CON. Both training groups increased 1-RM from pre to post (BFRRE 8.7 [3.8, 13.6]%; HLRE 19.6 [11.8. 27.4]%) and from pre to 14 days after cessation of training (BFRRE 9.90 [3.5, 16.3]%; HLRE 22.5 [14.1, 30.9]). Differences between BFRRE and HLRE were observed at post 4 and post 14. A tendency toward a difference between HLRE and CON was observed at post 4 ($p = 0.067$) and post 14 ($p = 0.072$).

Characteristics of Non-Responders and Responders

Given a large variability in the changes in muscle fiber CSA, and the additional insight gained by examining variable responses (Stec et al., 2016) we performed an analysis of responders versus non-responders to the current training regimen. Clustering of non-responders and responders were based on the magnitude of relative changes in total muscle fiber CSA (weighted mean of type I and II CSA). Mean changes in fiber CSA were −7.0 [−11.1, −2.89]% in non-responders and 13.3 [6.8, 19.8]% in responders (**Figure 9A**). A tendency toward lower fiber CSA and myonuclear domain (**Figures 9D,E**) were observed before the training intervention in the non-responders compared to responders (**Figures 9A,F**). Training led to an increase in number satellite cells per fiber in responders only (**Figure 9C**). Furthermore, RNA synthesis tended to be higher in responders compared to non-responders (**Figure 9B**). No differences between clusters were observed in myofibrillar MPS ($p = 0.36$) or total RNA content ($p = 0.92$) (data not shown).

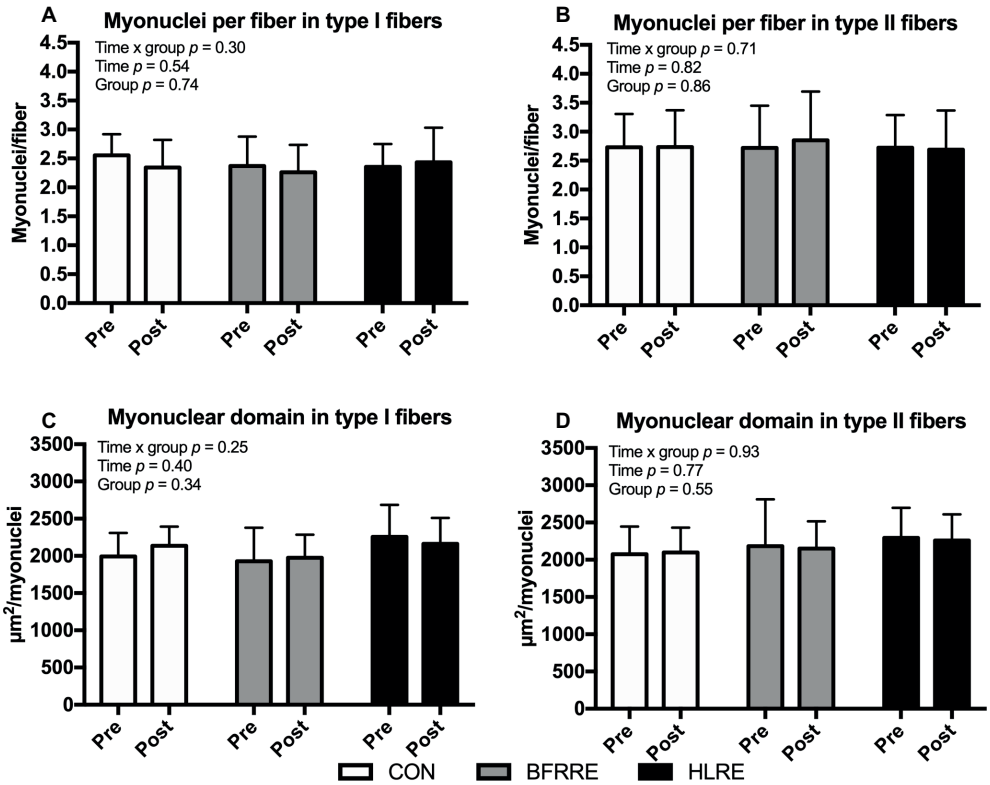

FIGURE 7 | Myonuclei at baseline (pre) and 4 days after cessation of training (post) expressed relative to number of fibers **(A,B)** and as myonuclear domain **(C,D)**. Data are presented as mean ± SD. Overall effects are given in the upper left corner of graphs.

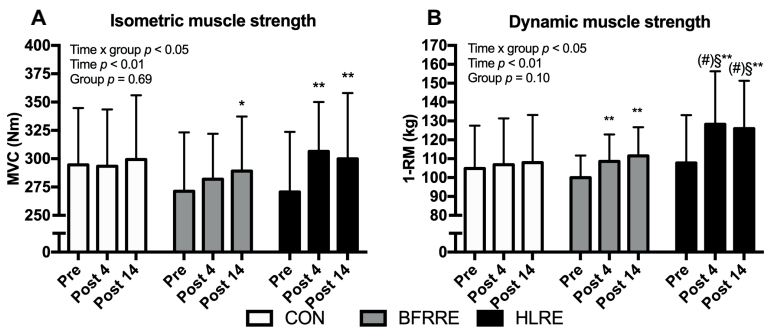

FIGURE 8 | Isometric muscle strength **(A)** and dynamic muscle strength **(B)** at baseline (pre), 4 days after cessation of training (post 4), and 14 days after cessation of training (post 14). Data are presented as mean ± SD. Overall effects are given in the upper left corner of graphs. *$p < 0.05$ and **$p < 0.01$ different from pre within group; $^{§}p < 0.05$ different from BFRRE within time-point; (#) $p < 0.1$ tendency toward difference to CON within time-point.

DISCUSSION

The present study comprehensively investigated skeletal muscle adaptive responses to 6 weeks of BFRRE or HLRE conducted by recommended exercise principles. The main findings were; (1) that BFRRE and HLRE produced similar increases in long-term myofibrillar MPS and RNA synthesis without concomitant increases in muscle fiber CSA; (2) that increases in satellite cell content was observed in responders, and; (3) that muscle strength increased with both training regimens, albeit to greater extent with HLRE. The present data support the potential of low-load BFRRE as an alternative training modality in clinical settings.

With regard to the training regimens, we deliberately chose a rather short training period. In accordance, the effectiveness of a ≤6-week resistance training period has previously been demonstrated as capable of producing muscle hypertrophy (Nielsen et al., 2012; Fahs et al., 2015; Holloway et al., 2018; Bjornsen et al., 2018a). Moreover, we utilized recommended exercise principles (i.e., BFRRE; low load, many repetitions, short inter-set recovery and HLRE; high load, few repetitions,

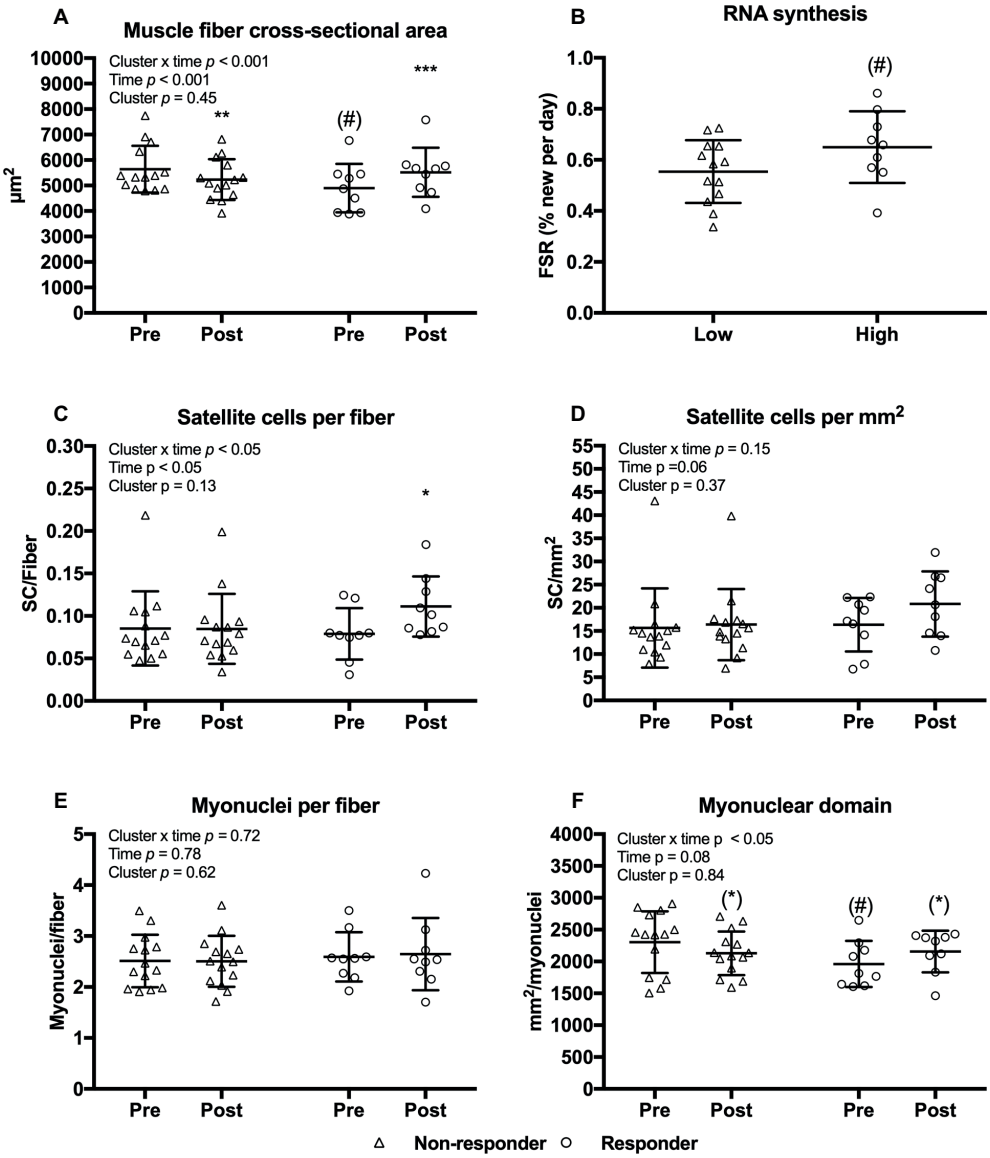

FIGURE 9 | Muscle fiber cross-sectional area **(A)**, satellite cells per fiber **(C)**, satellite cells per mm^2 **(D)**, myonuclei per fiber **(E)**, and myonuclear domain **(F)** at baseline (pre) and 4 days after cessation of training (post) in non-responders (n = 14) and responders (n = 9) with regards to muscle fiber hypertrophy. RNA synthesis in non-responders and responders **(B)**. Data are presented as individual values as well as mean ± SD. Overall effects are given in the upper left corner of graphs. (*)p < 0.1 tendency toward difference from pre within cluster. *p < 0.05, **p < 0.01, and ***p < 0.001 different from pre within cluster; (#)p < 0.1 tendency toward difference to non-responders within time-point.

long inter-set recovery; ACSM, 2009; Scott et al., 2015). This approach was chosen to resemble how exercise is practiced outside the laboratory rather than attempting to appoint one of the several differences between BFRRE and HLRE (i.e., ischemia, load, volume, and inter-set recovery) as more important for standardization.

Effects of BFRRE and HLRE on Myofibrillar Protein and RNA Synthesis

Previous studies utilizing short-term primed continuous amino acid infusions have reported that low-load BFRRE as well as HLRE can stimulate acute increases in myofibrillar MPS (Phillips et al., 1997; Fujita et al., 2007; Fry et al., 2010). A study by Burd et al. (2010) reported that mixed MPS was augmented at 24 h after single-bout low-load compared to high-load resistance exercise, but this study did not employ blood flow restriction. Notably, the MPS response to an acute bout of exercise can persist for ~24–72 h post-exercise (Phillips et al., 1997; Miller et al., 2005; Damas et al., 2016). The current study employed an approach using D_2O to assess cumulative myofibrillar MPS. This approach allows for assessment of long-term protein synthesis under free-living conditions and better accounts for proteins with slower turnover rates and/or less abundance

(Miller et al., 2015). Using this approach, we found an increase in cumulative myofibrillar MPS after HLRE, which is in accordance with recent studies using D_2O (Brook et al., 2015, 2016). Interestingly, low-load BFRRE produced a similar increase in myofibrillar MPS as HLRE. These findings support the notion that BFRRE provides a low-load approach to stimulate long-term protein turnover. This notion is further supported by our novel findings of increased cumulative RNA synthesis and increased total RNA content with BFRRE as well as HLRE. Since ~85% of total RNA exists as ribosomal RNA (Zak et al., 1967), increases in cumulative RNA synthesis and content observed by ourselves and others (Figueiredo et al., 2015; Brook et al., 2017) therefore primarily likely reflects increased ribosomal biogenesis and content, which may yield an increased translational capacity. In the current study, changes in RNA content were not correlated to RNA synthesis. This would suggest that rates of ribosomal biogenesis cannot quantitatively predict increases in ribosomal content and changes in ribosomal content is vice versa not indicate of how much new is made. As with protein content, ribosomal content is regulated by synthesis and breakdown. Ribosome breakdown was not assessed here, but we speculate that the observed increase in cumulative RNA synthesis was likely targeted for both ribosome accretion and ribosome maintenance/repair.

In both the current study, and the study by Brook et al. (2017), RNA synthesis and MPS were strongly correlated. This is further corroborated by studies reporting that *in vitro* protein synthesis is highly dependent on ribosomal content and biogenesis (Stec et al., 2016; West et al., 2016). Interestingly, RNA synthesis, but not RNA content, was correlated to MPS in our study which would suggest that the ability to increase MPS *in vivo* may preferentially be tied to the ability to make new ribosomes, rather than to increase the overall ribosome content On the other hand, previous human resistance training studies have advocated that changes in RNA content are in fact correlated to muscle hypertrophy (Figueiredo et al., 2015), which would naturally be preceded by increases in MPS. Thus, available evidence from our study and previous investigations strongly indicate that ribosomal biogenesis and MPS are involved in a coordinated regulation of protein turnover in response to exercise. However, we acknowledge that the utilization of a 6-week measurement period does not allow us to decipher whether the synthetic responses were primarily driven by large increases in the early phase of the training period, so this aspect warrants further investigation.

The heterogeneity of response between individuals to resistance exercise training is increasingly appreciated (Stec et al., 2016). Knowledge on the heterogeneity is important for appropriate exercise prescription as well as determining mechanisms that dictate muscle growth. A previous study from Bamman and co-workers stratified responders and non-responders to a resistance training protocol and demonstrated that an increase in ribosomal content was a key differentiating factor between the groups (Stec et al., 2016). In the current study, we did not observe a correlation between neither RNA synthesis nor RNA content and changes in muscle fiber CSA (R^2 = 0.09, p = 0.18; R^2 = 0.000, p = 0.87). However, when we performed a cluster analysis by pooling the subjects and separating responders from non-responders according to their individual change in muscle fiber CSA, there was a tendency (p = 0.098) toward a higher rate of RNA synthesis in responders. We observed no difference in RNA content between clusters but HLRE studies with a larger sample size than the current study have found that increases in RNA content are significantly greater in responders (Stec et al., 2016; Mobley et al., 2018). Ribosomal biogenesis therefore seems to constitute one underlying factor determining the muscle hypertrophic response to exercise.

To assess whether resistance training led to an increase in transcriptional capacity from satellite cell-mediated addition of myonuclei to existing muscle fibers, we analyzed satellite cell and myonuclei content. No changes in satellite cell or myonuclei content were observed. Only responders (as clustered based on relative change in muscle fiber CSA) showed a modest increase in satellite cell content. This suggests that under the current conditions, the transcriptional capacity of existing myonuclei was generally sufficient to provide mRNA transcript material for increased MPS [for further insight on this, we refer to Figueiredo and McCarthy (2019)].

Unlike the lack of change in satellite cell content in the current study, one previous study by Nielsen et al. (2012) reported remarkable increases in satellite cell content after BFRRE training. The Nielsen study also observed large increases in muscle fiber CSA. However, it should be noted that this study employed 23 exercise sessions in 19 days. In contrast, we employed 18 sessions over the course of 6 weeks, which obviously allowed for much more extended recovery between exercise sessions. The influence of recovery between BFRRE exercise sessions on satellite cells and fiber growth deserves further attention.

Increased Protein Turnover With BFRRE and HLRE May Reflect Muscle Remodeling

Based on previous D_2O studies showing correlations between cumulative increases in MPS and muscle hypertrophy (Brook et al., 2015; Damas et al., 2016), we hypothesized that an increase in MPS would lead to detectable muscle accretion as reflected by an increase in muscle fiber CSA. However, fiber-type-specific CSA as well as area-frequency distribution remained unchanged in all groups. This is in contrast to previous reports of increased muscle fiber CSA following BFRRE and HLRE training of comparable or even shorter duration than in our study (Staron et al., 1991; Goreham et al., 1999; Nielsen et al., 2012; Holloway et al., 2018; Bjornsen et al., 2018a,b). Nonetheless, it is debatable whether the low-volume single-exercise type of training, as employed in the current study, is sufficient to promote detectable muscle fiber hypertrophy after 6 weeks (McGlory et al., 2017) when also considering the large variation inherent of the single-biopsy technique (Lexell et al., 1985; Lexell and Taylor, 1989). Independent studies that have used ultrasound and magnetic resonance imaging (MRI) techniques, which have a generally high reliability in measuring whole muscle CSA (Franchi et al., 2018), have reported detectable hypertrophy following BFRRE as well as HLRE training (Kacin and Strazar, 2011;

Ellefsen et al., 2015; Sieljacks et al., 2018) of shorter or comparable length than ours. For instance, using MRI we recently found CSA increases of ~8% in VL and ~3% in *m. quadriceps* following a comparable BFRRE training intervention in a similar population (Sieljacks et al., 2018).

The finding that 6 weeks of BFRRE and HLRE training increased MPS without any changes in muscle fiber CSA suggests that BFRRE and HLRE may have stimulated muscle remodeling. We were not able to assess bulk changes in muscle protein breakdown with use kinetic stable isotope methodologies. To provide indicatory information on activation on protein degradation, we analyzed biomarkers of protein degradation signaling. FoxO3 signaling is involved in both proteasomal and autophagy-related gene expression (Zhao et al., 2007; Milan et al., 2015) for E3 ligase gene transcription inherent of the ubiquitin proteasome system which is activated during atrophy conditions. We did not observe changes in FoxO3 phosphorylation. Autophagy constitute a catabolic process known to enable cellular remodeling by delivering dysfunctional proteins and organelles to the lysosomes for degradation (Boya et al., 2013; Bell et al., 2016). In previous studies, it was shown that an acute bout of exercise stimulates autophagy signaling through ULK1 in human skeletal muscle (Moller et al., 2015, 2018). Animal studies support that exercise stimulate increases in autophagy flux and that this activation is necessary to attain training adaptations. Consequently, our data support that autophagy signaling through ULK1 is stimulated during an acute bout of BFRRE and HLRE to engage in remodeling processes. However, it needs to be emphasized that our data constitute merely markers of degradation as no method exist to assess autophagy flux in human tissues *in vivo*. Moreover, we acknowledge that other biomarkers of protein degradation pathways (such as calpain and ubiquitin proteasomal systems) and/or other time points of measurement must be included in future studies, to provide for strengthened conclusions. Finally, it should be acknowledged that subjects were not accustomed to the exercise stimuli prior to the single-bout trial, which may likely affect signaling outcome (Wilkinson et al., 2008). However, our results support the notion that BFRRE and HLRE, increased protein turnover by stimulation of muscle MPS and muscle protein degradation. The ability of BFRRE to presumably stimulate muscle protein turnover may emphasize the potential application of BFRRE in ageing and disease settings even though no hypertrophy was observed, as protein turnover is critical during ageing and disease to prevent accumulation of damaged proteins (Haus et al., 2007; Gouveia et al., 2017; Musci et al., 2018). Noteworthy, the current study was made on healthy subjects and future studies should explore these responses in clinical populations.

Effects of BFRRE and HLRE on Muscle Functional Capacity

HLRE produced an increase in MVC as well as maximal dynamic strength measured at both 4 and 14 days after cessation of training. Similar increases were produced with BFRRE. Yet, the increase in MVC with BFRRE did not reach statistical significance until 14 days after training. Similar observations of delayed maximal strength gains after BFRRE training have been reported previously (Nielsen et al., 2012), and it was to be owing to impaired intrinsic muscle function (Nielsen et al., 2017) Alternatively, the strength increase at post 14, could potentially relate to a learning effect between testing sessions.

The apparent greater ability of HLRE to enhance maximal strength has also been reported in other comparative studies (Karabulut et al., 2010; Yasuda et al., 2011; Martín-Hernández et al., 2013). The greater strength gains with HLRE may relate to differential neural innervation patterns/adaptations between BFRRE and HLRE. HLRE has been shown to induce greater EMG-assessed muscle activation during acute exercise compared to BFRRE (Cook et al., 2013), indicating greater motor unit recruitment and/or firing rates (Suzuki et al., 2002). Similarly, long-term HLRE has been shown to increase muscle activation following long-term training while no changes occurred following BFRRE (Kubo et al., 2006). It therefore seems that HLRE constitutes a stronger driver of maximal strength than BFRRE in young healthy subjects.

CONCLUSION

The current study demonstrates that BFRRE and HLRE stimulate protein turnover, RNA synthesis, and increase muscle strength. These adaptations could be beneficial during ageing and disease to maintain protein homeostasis, muscle mass, and mobility. Owing to the low load, BFRRE may constitute a feasible and time-efficient training modality for certain clinical populations. Future studies should investigate the use of BFRRE in such populations.

ETHICS STATEMENT

Written informed consent was obtained from all participants prior to inclusion. The study was approved by the Central Denmark Region Committee on Health Research Ethics (1-10-72-218-16) and registered in the database clinicaltrials.gov (NCT03380663). The study conformed to the standards for human experimental trials outlined in the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

The study was conducted at Section for Sports Science, Department of Public Health, Aarhus University. PS, TG, ER, FP, KH, BM, and KV contributed to conception and design. All authors contributed to data acquisition and/or interpretation of data. PS and KV wrote the first manuscript draft. All authors critically revised the manuscript and provided

intellectual contributions. All authors approved the final version of the manuscript submitted for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/article/10.3389/fphys.2019.00649/full#supplementary-material

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Progression of Blood Flow Restricted Resistance Training in Older Adults at Risk of Mobility Limitations

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Blood flow restriction (BFR) resistance training leads to increased muscle mass and strength but the progression leading to adaptations may be different as strength gains are often to a lesser magnitude than high-load (HL) training. The impact of training loads and repetitions on older adults' muscle mass and strength following BFR or HL training was evaluated. Twenty-one older adults (67–90 years) classified as being at risk of mobility limitations were randomly assigned to HL (n = 11) or BFR (n = 10) knee extension (KE) and flexion (KF) training twice per week for 12 weeks. Strength was measured with 10-repetition maximum (10-RM) tests and isometric contractions. Cross-sectional area (CSA) of the quadriceps and hamstrings was measured. HL and BFR interventions increased 10-RM KF and isometric strength ($P < 0.05$) and hamstrings CSA increased an average of 4.8 ± 5.9% after HL and BFR training (time main effect $P < 0.01$). There were no differences between the training groups (time x group interactions $P > 0.05$). The rate of progression of KF training load and repetitions was comparable (time × group interactions of each variable $P > 0.05$). The groups averaged an increase of 0.50 ± 25 kg·week^{-1} and 1.8 ± 0.1.7 repetitions·week^{-1} of training (time main effects $P < 0.05$). The HL training group experienced greater improvements in KE 10-RM strength than the BFR group (60.7 ± 36.0% vs. 35.3 ± 25.5%; $P = 0.03$). In both groups, isometric KE strength increased 17.3 ± 18.5% ($P = 0.001$) and there were no differences between groups ($P = 0.24$). Quadriceps CSA increased (time main effect $P < 0.01$) and to similar magnitudes (time x group interaction $P = 0.62$) following HL (6.5 ± 3.1%) and BFR training (7.8 ± 8.2%). The HL group experienced accelerated progression of load when compared to BFR (0.90 ± 0.60 kg·week^{-1} vs. 30 ± 0.21 kg·week^{-1}; $P = 0.006$) but was not different when expressed in relative terms. BFR training progressed at a rate of 3.6 ± 1.3 repetitions·week^{-1} while the HL group progressed at 2.2 ± 0.43 repetitions·week^{-1} ($P = 0.003$). HL training led to greater increases in KE 10-RM and it may be attributed to the greater load and/or faster rate of progression of the load throughout the 12-week training period and the specificity of the testing modality. Incorporating systematic load progression throughout BFR training periods should be employed to lead to maximal strength gains.

Keywords: resistance training, progression, older adults, blood flow restriction, hypertrophy

Abbreviations: BFR, blood flow restriction; CSA, cross sectional area; HL, high-load; KE, knee extension; KF, knee flexion; RM, repetition maximum.

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INTRODUCTION

Skeletal muscle adaptations of muscular hypertrophy, strength, power, and endurance can be obtained through resistance training programs that specifically alter the load, sets, repetitions, and exercise volume (Garber et al., 2011). Resistance training utilizing moderate to HLs of 60–80% of an individual's one-repetition maximum (1-RM) with 2–4 sets of 8–12 repetitions has been shown to be effective for improving muscle hypertrophy, strength and power in healthy young, and older adults while muscle endurance adaptations are achieved using low-load resistance training that incorporates less than 50% 1-RM for 2–4 sets of 15–20 repetitions (Garber et al., 2011). To elicit these adaptations, the principles of specificity, progression, and overload are incorporated over time (Ciolac et al., 2010). Progression and overload can be achieved through an increase in the number of repetitions, the load used, frequency of training sessions per week, or the volume of the resistance exercises (Kraemer and Ratamess, 2004). Specificity is achieved through the appropriate muscles involved, the movement pattern and the contraction type within the training program (Haff and Triplett, 2016).

It is recommended that older adults engage in moderate to HL resistance training protocols to combat sarcopenia with the intention to preserve physical function (Aagaard et al., 2010; Peterson et al., 2010) however, the high mechanical loads and stresses may be contraindicated due to a greater risk of injury and the greater prevalence of musculoskeletal disorders in older adults (Blyth and Noguchi, 2017). Therefore, it is important to explore alternate modalities other than HL resistance training for older adults to engage in to maximize muscle size, strength, power, and endurance.

Blood flow restricted (BFR) exercise incorporates the use of lighter loads and more repetitions than HL training. BFR exercise intervention studies or clinical trials typically last 6–12 weeks and focus on maximizing adaptations of increased muscle mass and strength (Lixandrão et al., 2018). Loads used in this exercise training are approximately 20–30% of 1-RM and participants perform multiple sets of 15–30 repetitions (Loenneke et al., 2012b; Pope et al., 2013). BFR exercise does result in improved muscle mass and strength in young adults (Laurentino et al., 2012; Martín-Hernández et al., 2013; Ellefsen et al., 2015) and older adults (Takarada et al., 2000; Karabulut et al., 2010; Vechin et al., 2015) and the adaptations have been shown to be comparable to, but not greater than the adaptations seen from HL training in both younger and older adults (Loenneke et al., 2012b; Martín-Hernández et al., 2013; Vechin et al., 2015; Cook et al., 2017). The proof of concept that BFR resistance training is an effective exercise modality has been supported. Since it may not be as effective as HL resistance training, researchers should focus on altering variables, such as cuff types, restriction pressures, loads, sets, repetitions, and volume to maximize adaptations. Considerable research has been done on BFR cuff type and restriction pressure leading researchers to summarize that restriction pressure should be relative to the cuff width and individualized to limb circumference (Mattocks et al., 2018). While studies have summarized the literature on load, repetitions, and volume (Scott et al., 2015) there are no guidelines or recommendations on the progression of BFR exercise throughout the course of a training period.

BFR exercise is an attractive clinical exercise modality for young and older adults due to the reduced stress placed on the musculoskeletal system, thus allowing individuals with mobility limitations and those with disorders or injuries to participate (Segal et al., 2015; Bryk et al., 2016; Hughes et al., 2017; Ladlow et al., 2018). Our previous research demonstrated the effectiveness of BFR resistance exercise in older adults with mobility limitations (Cook et al., 2017) and this secondary research study investigates the progression of the participants' training loads, repetitions and volume during the 12-weeks of HL and BFR resistance training. It was speculated that the rate of change in loads and repetitions over a training period would vary between HL and BFR training which could describe the consistent findings that strength gains after BFR training are rarely greater than HL strength gains.

MATERIALS AND METHODS

Experimental Design

A between groups repeated measures design was used to assess exercise loads and repetitions, muscle strength and CSA of older adults before and after 12-weeks of a resistance training exercise intervention. A stratified randomization approach was used to place participants by age (65–75 years and 75+ years) and sex into one of two resistance exercise interventions: HL or BFR and an attention control condition. The data for the control group is not reported in this study but can be found in a previous publication (Cook et al., 2017).

Subject Recruitment and Participant Descriptions

Community dwelling males and females $\geq$65 years old were recruited to participate in this study. The recruitment approach and inclusion criteria are described in detail elsewhere (Cook et al., 2017). Briefly, older adults that were classified as at risk of mobility limitations (Manini et al., 2007) and met the health and strength criteria volunteered for the study. All participants signed an informed consent approved by the University of New Hampshire Institutional Review Board to participate in the resistance training study. The volunteers gained approval from a primary care provider to participate and underwent an exercise stress test on a treadmill supervised by a cardiologist that also provided medical clearance. These individuals then underwent a familiarization session in which they were orientated to the exercise equipment, the strength testing protocol, and the general study procedures. The short physical performance battery (SPPB) was done to assess the participants' abilities to complete chair stands, balance tests and a 4-m walk. The SPPB scores range from 0 to 12; with 12 indicating the highest degree of lower extremity function. Participants were then randomly assigned to the HL, BFR or attention control condition. Twenty-one participants (9 males and 12 females; **Table 1**) aged 67–92 years old fully completed the study and their data were used in the data analysis.

Exercise Intervention

The participants underwent 12-weeks of twice per week supervised resistance training using seated KE and knee flexion (KF) machines (Body Solid GCEC340, Forest Park, IL, United States). A horizontal leg press machine (Body Solid GLP-STK, Forest Park, IL, United States) was also used in the training but this data was not used in the analysis due to the infrequency of training on this equipment in BFR exercise literature. Each exercise session consisted of a warm-up of 10 repetitions at a very light weight (∼5% of 1-RM) and progressed into three sets of each exercise performed to volitional failure with 60 s of rest between sets and 3 min between exercises. Volitional failure occurred when the individual could not complete full range of motion or ceased exercise due to perceived fatigue. Ratings of perceived exertion (RPE) using a 1–10 scale were provided by participants after each set of exercise. The participants performed only one set of each exercise for the first week, two sets the second week, and three sets for the remainder of the study. The participants assigned to the HL intervention performed the lower body exercises listed above at 70% of an estimated 1-RM. The concentric and eccentric portions of the exercise movement lasted 3 s (a rate of 20 contractions per minute) and were controlled by a metronome. Exercise load was progressed by 1–2 kg when participants were able to perform more than 15 repetitions for at least two sets of exercise on a given day. Participants assigned to the BFR training group performed the KE and KF exercise at 30% of estimated 1-RM while the leg press exercise was performed at 50% of estimated 1-RM. The load of 50% was chosen due to inability to restrict blood flow to the gluteal muscles involved in this exercise. The BFR was applied to the proximal portion of the leg utilizing a narrow (6 cm × 83 cm) pneumatic tourniquet cuffs (D.E. Hokanson, Inc., Bellevue, WA, United States) that were inflated before exercise (Hokanson TD312 Calculating Cuff Inflator, Bellevue, WA, United States). The cuff was set at approximately 1.5 times brachial systolic blood pressure which equated to an average pressure of 184 ± 25 mmHg applied to the participants. Based on a previous study that predicted arterial occlusion pressure from systolic and diastolic blood pressure and thigh circumference (Loenneke et al., 2015) the BFR pressure used in our study was approximately 66% of predicted arterial occlusion pressure. This percentage is similar to other studies (Laurentino et al., 2012; Soligon et al., 2018). The BFR cuffs remained inflated during each exercise and the rest between sets. This resulted in approximately 5 min of restriction time per exercise. The cuffs were deflated for the 3-min rest periods between exercises. Exercise load was progressed by approximately 1–2 kg when participants were able to perform more than 30 repetitions for at least two sets of exercise on a given day. Load, repetitions and exercise volume (load × repetitions) were recorded daily for all participants in the HL and BFR training interventions. The average rates of progression in load, repetitions, and volume per month were calculated from the difference in weeks 1–4, 4–8, and 8–12.

TABLE 1 | Descriptive data presented as mean (standard deviation) of the exercise intervention groups.

	HL	BFR
N	11 (5M, 6F)	10 (4M, 6F)
Age (years)	76.3 (8.7)	76.4 (6.6)
Mass (kg)	73.3 (10.9)	75.4 (10.9)
Height (m)	1.7 (0.1)	1.7 (0.1)
Body mass index (kg·m^2)	26.5 (3.0)	27.5 (3.3)
Short physical performance battery (SPPB)	10.7 (2.1)	10.2 (1.9)

There were no differences between the groups ($P > 0.05$).

Measurements

Strength and CSA were assessed prior to the intervention and at 12-weeks of training. The testing was spread out over 4 days that included: a magnetic resonance imaging (MRI) scan to determine CSA; strength testing on the dynamometer and 10-RM testing. The post testing began 2–4 days after the last exercise training session.

Cross-Sectional Area

The average CSA of the quadriceps and hamstrings muscle groups on the right leg were obtained through serial axial MRI scans. CSA was obtained from the upper leg (greater trochanter to patella) using a 1.5-T Phillips Intera whole body scanner with software Release 11 (Phillips Medical Systems, Bothell, WA, United States). Ten mm-thick transaxial images (2122-ms repetition time, 10.12-mm slice-to-slice interval) were taken after a 30-min supine rest period to allow for fluid equilibration. The images were transferred to a computer for calculation of muscle CSA using the National Institutes of Health ImageJ software (Abramoff et al., 2004). A research technician was blinded to participants' group assignments and time points of MRI scan. To calculate CSA (cm^2) of the quadriceps and hamstrings all the images were traced from the appearance of the distal portion of the rectus femoris to the appearance of the femoral neck. The same number of slices using the anatomical landmarks was measured for each subject at each of the testing time points (∼10 ± 1 images). Measurements were performed in duplicate and the average CSA of the quadriceps and hamstrings were used in the analysis. The test-retest reliability of CSA of the quadriceps was previously determined to have an intraclass correlation coefficient (ICC) of 0.99 (Cook et al., 2010).

Strength

Unilateral, isometric maximum voluntary contraction (MVC) torque at 60°; of extension (0° was full extension) was assessed on the right knee extensors and flexors on an isokinetic dynamometer (HUMAC NORM, CSMI, Stoughton, MA, United States). Participants were instructed to produce as much force, as quickly as possible, for a 3-s maximal contraction following an auditory cue. Three trials were performed, and the last two contractions were averaged. The trial-to-trial ICC of the MVC was 0.99.

Bilateral 10-RM was determined using the 10-RM approach. Briefly, participants performed a light warm-up on the KE, KF, and LP machines. Load was progressively increased until participants could perform approximately 10 repetitions. 1-RM

was then predicted using an equation (Haff and Triplett, 2016) to set the exercise load for the resistance training protocols (70% of 1-RM for HL training and 30% of 1-RM for BFR training). Predicted 1-RM from the 10-RM test was also used to further adjust workout loads after 6-weeks of training.

Statistical Analyses

The sample size for this secondary analysis study was dependent on our previous study (Cook et al., 2017). Data are expressed as mean ± standard deviation. Repeated measures analysis of variance (ANOVA) procedures were used to detect differences in the dependent variables (10-RM and MVC in KE and KF, CSA in hamstrings and quadriceps, and exercise repetitions, loads and volumes) performed by the participants with respect to the within-subjects independent variable (pre and post-training) and the between subjects training factor (HL and BFR). Significant interactions and main effects were followed with appropriate *post hoc* tests, including Tukey *post hoc* tests or *t*-tests with Bonferroni adjustments. Relative changes were calculated as pre values divided by post values and expressed as a percentage. Independent *t*-tests were used to assess the relative changes between training interventions. An alpha level of 0.05 was required for statistical significance. IBM SPSS Statistics version 24.0 (Chicago, IL, United States) was used to analyze the data.

RESULTS

The participants placed in the HL and BFR training interventions were of similar age, mass, height, body mass index, and physical function upon entry into the study ($P > 0.05$; **Table 1**). There was 100% compliance in the 24 exercise sessions completed by both the HL and BFR training groups. Systolic and diastolic blood pressure values before and after the training did not change (time main effect $P > 0.05$ for both variables, $\eta^2 < 0.06$; time x group interactions $P > 0.05$, $\eta^2 < 0.03$). No adverse events occurred during the study.

Cross-sectional area of the quadriceps increased 6.5 ± 3.1% and 7.8 ± 8.2% in the HL and BFR training groups, respectively (**Table 2**). There was not a time × group interaction ($P = 0.86$, $\eta^2 = 0.01$) or group main effect ($P = 0.65$, $\eta^2 < 0.01$) but there was an overall main effect of time ($P < 0.01$, $\eta^2 = 0.66$) as the growth in CSA was significant. A similar trend was evident in the CSA of the hamstrings as the HL and BFR training groups improved 5.3 ± 7.4% and 4.8 ± 5.9%, respectively. There was a main effect of time ($P = 0.003$, $\eta^2 = 0.38$) and the time x group interaction ($P = 0.62$, $\eta^2 < 0.01$) and group main effect ($P = 0.86$, $\eta^2 = 0.01$) were not significant.

The HL training group experienced a 60 ± 33% improvement in 10-RM in the KE exercise while the BFR training group had a 36 ± 28% increase (time x group interaction $P = 0.02$, $\eta^2 = 0.25$; **Table 2**) however, there were no differences between the groups in the magnitude of strength gains in the KF 10-RM (time × group interaction $P = 0.11$, $\eta^2 = 0.13$). Both groups experienced increases in KE MVC and KF MVC (time main effect $P < 0.01$ for both variables, $\eta^2 = 0.47$ and 0.40, respectively; time x group interactions $P > 0.05$, $\eta^2 < 0.10$ **Table 2**).

Both groups combined demonstrated significant progression in KE and KF loads, repetitions and volume throughout the 12-week training period (time main effect $P < 0.01$ for all variables; $\eta^2 > 0.53$). There were significant group main effects for KE and KF loads ($P < 0.01$; $\eta^2 > 0.40$) as the load was always higher in the HL group than the BFR group. KE and KF repetitions ($P < 0.01$; $\eta^2 > 0.70$) in the BFR group was always higher than the HL group (**Figures 1C**, **2C**). Despite this, there were no group differences in KE volume ($P = 0.20$; $\eta^2 = 0.08$) and KF volume ($P = 0.14$; $\eta^2 = 0.11$) (**Figures 1**, **2**). There were significant time x group interactions in KE load ($P < 0.01$; $\eta^2 = 0.27$) and KE repetitions only ($P < 0.01$; $\eta^2 = 0.29$) (**Figures 1A,B**).

The KE load in the HL training group was always higher at weeks 1 through 12 than the BFR training group ($P < 0.01$; $\eta^2 = 0.47$) while the BFR training group always performed more repetitions than the HL training group (**Figures 1A,B**). Overall, the HL group averaged an increase of 10.80 ± 7.11 kg from the beginning to the end of the study (0.90 ± 0.60 kg·week^{-1}) in the KE exercise. This was significantly greater than the BFR group as they averaged 3.6 ± 2.6 kg (0.30 ± 0.21 kg·week^{-1}); ($P < 0.01$; **Table 3**). However, these differences disappeared when they were expressed in relative terms as percent change (**Table 3**) as both groups combined averaged a 40.6 ± 29.5% and 192.5 ± 74.9% increase in KE load and repetitions, respectively. The KE load in the HL training group was lowest within the first four weeks of

TABLE 2 | Muscle strength and mass measurements before and after high-load (HL) and blood flow restricted (BFR) training presented as mean (standard deviation).

	HL			BFR		
	Pre	Post	% change	Pre	Post	% change
Knee extension						
10-RM (kg)†	39.0(17.8)	60.5(25.3)	59.9(33.0)	36.6(17.6)	47.1(20.0)	35.8(27.8)
MVC (Nm)*	103.8(36.1)	126.0(42.5)	23.1(13.4)	115.9(38.5)	128.0(45.4)	11.0(21.9)
Quadriceps CSA (cm^2)*	44.7(11.7)	47.7(11.7)	6.5(3.1)	45.4(11.7)	48.9(13.2)	7.8(8.2)
Knee flexion						
10-RM (kg)*	26.0(10.3)	34.8(8.0)	40.4(28.3)	28.5(10.2)	33.2(12.6)	18.4(22.5)
MVC (Nm)*	60.7(18.4)	68.9(23.0)	13.7(14.5)	63.4(19.6)	69.5(19.3)	11.9(17.3)
Hamstrings CSA (cm^2)*	22.6(7.4)	23.5(7.1)	5.3(7.4)	21.3(6.0)	22.1(6.2)	4.8(5.9)

**Indicates significant main effect of timepoints for the overall sample ($P < 0.05$).* †*Indicates significant time x group interaction ($P < 0.05$).*

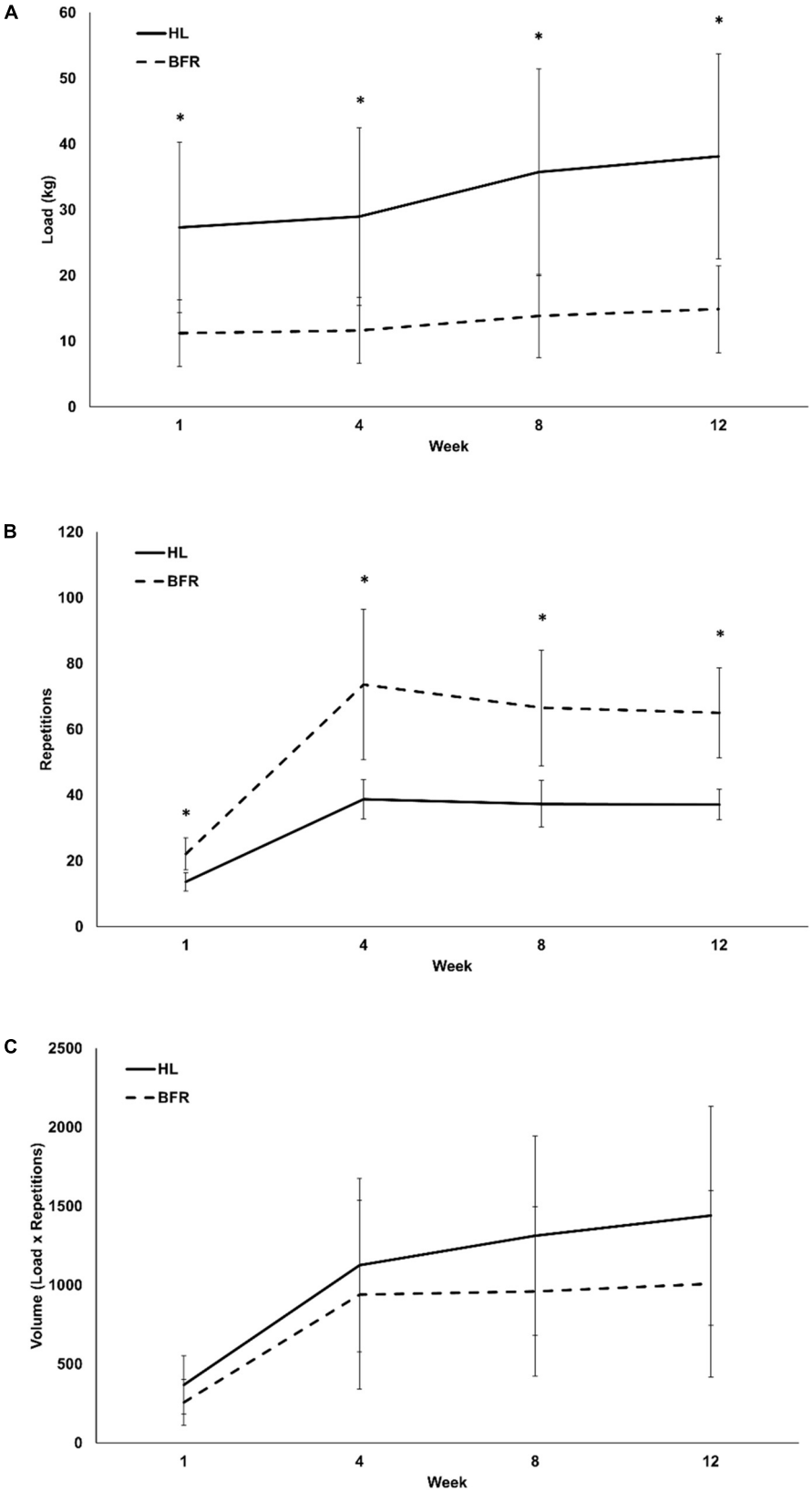

FIGURE 1 | Monthly load **(A)**, repetitions **(B)**, and volume **(C)** of high-load (HL) and low-load blood flow restricted (BFR) knee extension resistance training. * denotes significant difference between HL and BFR.

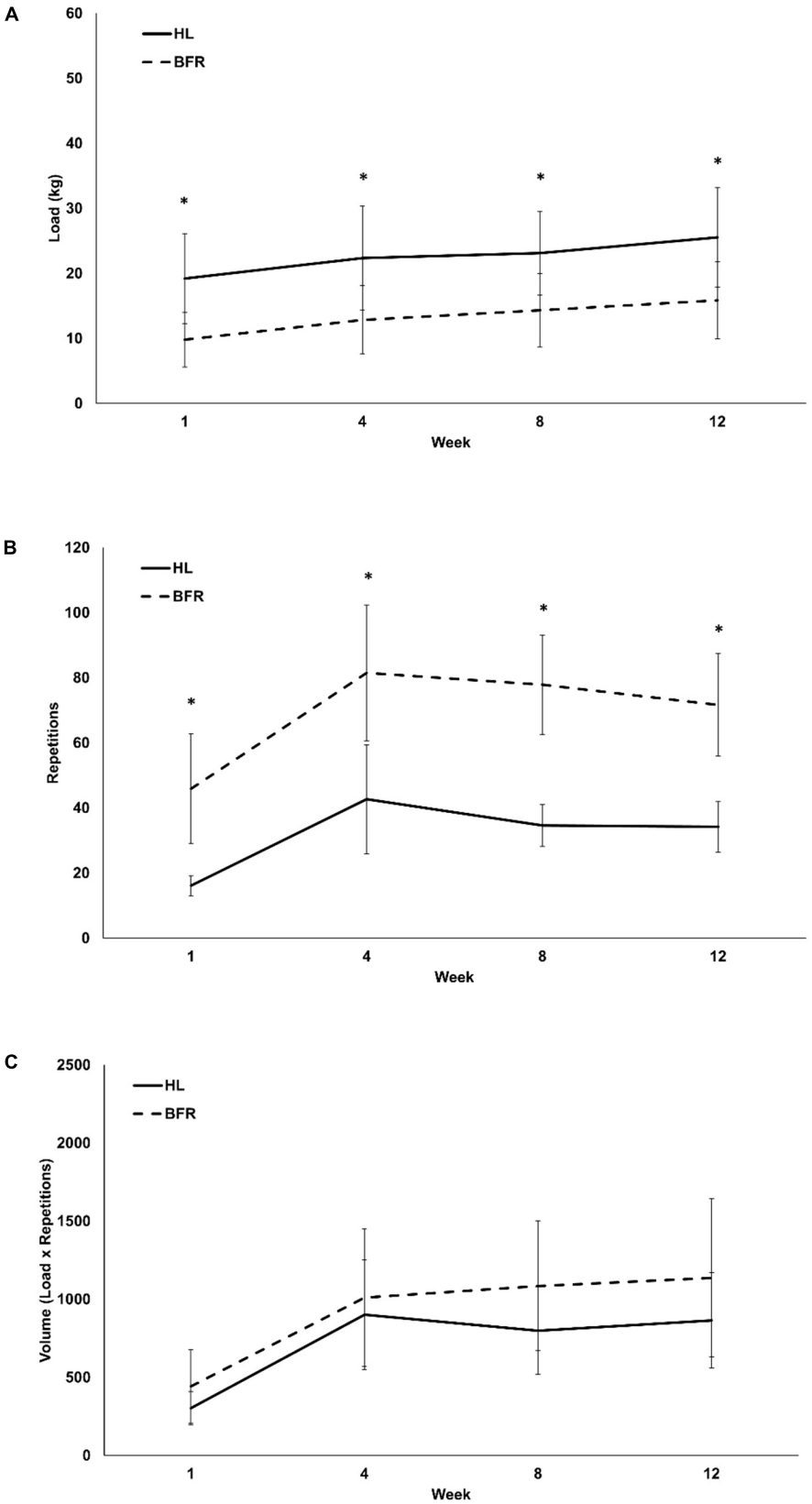

FIGURE 2 | Monthly load **(A)**, repetitions **(B)**, and volume **(C)** of HL and low-load blood flow restricted (BFR) knee flexion resistance training. * denotes significant difference between HL and BFR.

TABLE 3 | Absolute (kg) and relative (%) differences between load, repetitions and volume between weeks 1 and 12 in HL and blood flow restricted (BFR) training.

	HL		BFR	
	Absolute (kg)	**Relative (%)**	**Absolute (kg)**	**Relative (%)**
Knee extension				
Load (kg)	10.8(7.1)*	46.1(35.1)	3.6(2.6)	34.6(22.0)
Repetitions	23.5(5.0)*	181.3(63.7)	42.9(14.0)	204.8(87.5)
Volume (kg)	1072.0(558.1)	195.6(82.1)	750.7(511.7)	190.3(129.6)
Knee flexion				
Load (kg)*	6.3(1.7)	35.7(13.7)*	6.0(2.9)	67.4(41.2)
Repetitions	18.1(8.3)	120.7(69.5)	25.7(29.2)	78.7(78.4)
Volume (kg)	561.1(235.1)	305.7(118.6)	694.6(476.2)	312.5(135.1)

*Data are presented as mean (standard deviation). *Indicates significant difference from BFR ($P < 0.05$).*

training when compared to week 8 and it continually increased up until week 12 ($P < 0.01$). The KE load in the BFR training group was also lowest within the first four weeks of training compared to weeks 8 and 12, however, there were no further increases in KE load from weeks 8 to 12 ($P = 0.25$) (**Figure 1A**).

The BFR group completed more repetitions in the KE than the HL training group (3.6 ± 1.3 repetitions·week^{-1} vs. 2.20 ± 0.43 repetitions·week^{-1}; $P < 0.01$; **Figure 1B**). The HL and BFR training groups significantly increased the repetitions performed from week 1 to weeks 4, 8, and 12 ($P < 0.01$), but the repetitions were constant from weeks 4 to 12 ($P > 0.05$). KE exercise volume (**Figure 1C**) increased over the 12 weeks for both groups combined (time main effect $P < 0.01$; $\eta^2 = 0.71$) but there were no time x group interactions for KE volume ($P = 0.44$; $\eta^2 = 0.07$). RPE in the KE exercise was similar between the HL and BFR training groups ($P = 0.52$; $\eta^2 = 0.04$) but on average increased significantly from the week 1 (6.0 ± 2.0) to weeks 4, 8, and 12 (8.0 ± 2.0) (time main effect $P < .01$; $\eta^2 = 0.66$).

Over the 12 weeks of training there were significant increases in KF load ($P < 0.01$; $\eta^2 = 0.69$; **Figure 2A**), repetitions ($P < 0.01$; $\eta^2 = 0.53$; **Figure 2B**), and volume ($P < 0.01$; $\eta^2 = 0.71$; **Figure 2C**). The absolute change in KF load from the first to the twelfth week was not different between the training interventions (HL: 6.3 ± 1.7 kg or 0.53 ± 0.14 kg·week^{-1}, BFR: 6.0 ± 2.9 kg, or 0.50 ± 0.50 kg·week^{-1}, $P = 0.77$). However, when expressed as a percent of the first week of training, the BFR group experienced a 64.7 ± 41.2% increase in KF load while the HL group had an overall increase of 35.7 ± 13.7% ($P = 0.03$; **Table 3**). KF repetitions were not different between HL (18.1 ± 8.3 repetitions or 1.5 ± 0.69 repetitions·week^{-1}) and BFR (25.7 ± 29.2 repetitions or 2.1 ± 2.4 repetitions·week^{-1}) ($P = 0.42$; **Table 3**). RPE in the KF exercise was similar between the HL and BFR training groups ($P = 0.17$; $\eta^2 = 0.09$) but on average increased significantly from the week 1 (6.0 ± 2.0) to weeks 4, 8, and 12 (8.2 ± 1.7) (time main effect $P < .01$; $\eta^2 = 0.75$).

DISCUSSION

The findings from this study indicate that how exercise loads and repetitions are progressed likely have an impact on the muscular adaptations following resistance training in older adults. Increases in muscle mass and strength were evident in older adults at risk of mobility limitations following similar volumes of HL and BFR resistance training on the KE and KF muscle groups. However, the disparate strength gains in KE 10-RM in the HL training group may be due to multiple factors that could affect how researchers and clinicians implement and evaluate BFR exercise.

The similar gains in muscle mass following HL and BFR resistance training align with previous research on young adults (Laurentino et al., 2012; Martín-Hernández et al., 2013; Ellefsen et al., 2015) and older adults (Vechin et al., 2015). Even though no statistically significant differences were evident in strength gains in our study, we should consider that the percent change in KE 10-RM, KF 10-RM, and KE isometric MVC strength gains in HL training were approximately double than levels after BFR training. These diverse magnitudes of strength gains in the KE following BFR training have been reported in other studies in young adults (Martín-Hernández et al., 2013) and older adults (Karabulut et al., 2010; Vechin et al., 2015) and has been further described in a recent meta-analysis (Lixandrão et al., 2018). The comparable levels of KE muscle hypertrophy and disparate gains in KE strength imply that neural factors may play a key role. It also highlights the premise that preserving and gaining muscle mass in older adults may not be enough to impact physical function (Kim et al., 2012) and we must continue to optimize resistance training since it is the primary treatment for sarcopenia (Liguori et al., 2018).

One such component to further explore is the rate of change in KE exercise load during HL and BFR training. Despite having a lower rate of change in the number of repetitions performed when compared to BFR training, HL training had a much greater absolute change in load while volume in both training protocols remained constant. Our research quantifies the rate of progression in the KE exercise based on changes in load throughout the 12-week training duration of the study. The participants in the HL group experienced weekly increases in load of approximately 0.90 ± 0.60 kg·week^{-1} as they began with an average training load of 27 kg and progressed to 38 kg in the final week of training. The rate of progression in the HL group was three times greater than

the BFR training group. Our previous publication using the same sample of participants demonstrated that most strength gains from HL and BFR exercise were obtained within the first 6-weeks of the resistance training program and only the HL group continued to have significant gains in the KE from weeks 6 to 12 (Cook et al., 2017). One of two situations may be possible based on the results of our research. First, it is reasonable to assume that the strength benefits from BFR resistance exercise are gained within the first few weeks of resistance training after which further improvements can only occur through HL resistance training. This aligns with the clinical application of utilizing BFR resistance exercise in patients undergoing bed rest and progressing them to HL resistance training (Loenneke et al., 2012a). The second situation may be that the progression of BFR KE exercise focuses more on loads rather than repetitions. For example, in our study the BFR group began training at an average load of 11 kg and progressed at a rate of 0.30 ± 0.21 kg·week^{-1} to a 15 kg load at the final week of training. If the BFR training group progressed at the same rate as the HL training group, their final load would be approximately 22 kg which would still be considered a low load of 46% 1-RM. Interestingly, when expressing these differences in load and repetitions in relative terms based on percent change, these differences disappear. Irrespective of how the changes in sets and repetitions are described, consideration of resistance exercise progression in older adults deserves future investigation.

It is well known that resistance exercise at high as well as low loads lead to enhanced strength in novice resistance trainers mainly due to improvements in motor learning and coordination (Rutherford and Jones, 1986; Kraemer and Ratamess, 2004). Further neuromuscular adaptations then arise from enhanced motor unit recruitment, rate coding and synchronization that may require loads of 80–85% of 1-RM to result in strength gains in advanced resistance trainers (Kraemer and Ratamess, 2004). In our study, the participants in the HL training group experienced significant increases in KE load throughout the entire 12-weeks of the study while the BFR training group had significant increases in load only within the first 4-weeks of the study. This difference in training progression may be an area for future studies to control for when implementing BFR resistance training protocols. It should also be considered that the 10-RM strength tests used loads most similar to those employed in the HL training program and as a result, 10-RM testing may have been more sensitive to the strength improvement of HL training than the BFR training that was performed at lighter loads. Researchers have suggested the use of isometric MVC strength as a neutral test to assess effectiveness of resistance training protocols (Buckner et al., 2017). As such, in the present study there were improvements in KE MVC following HL and BFR training (23 ± 13% and 11 ± 22%, respectively).

It is interesting that the KF muscle group did not have as robust adaptations following HL and BFR training as the KE muscle group exhibited, despite the reporting of similar RPE values. We suspect that this may be due partly to the differences in neuromuscular properties within the lower limb muscles as it has been suggested that extensor motor neurons are more plastic and adapt more readily to activity-based interventions (Kirk et al., 2018). Nevertheless, the KF muscle group plays an important role in the gait cycle and deserves more attention in older adults. There is limited data on the effects of BFR resistance exercise on KF strength and hamstring CSA. To our knowledge, only two studies have evaluated this muscle group and the BFR exercise was done during rehabilitation from knee surgery. Ohta et al. (2003) reported a milder atrophy and strength recovery after surgery when compared to the heavily impacted quadriceps that lost significant muscle mass and strength. Similarly, Tennent et al. (2017) reported a 77% and 39% increase in KE and KF muscle strength, respectively following 12 sessions of postoperative physical therapy. The magnitude of change in the KE and KF were more than twofold increases when compared to the conventional therapy offered to the control group (Tennent et al., 2017). Our study noted similar, significant improvements in KF strength after both training programs. While the absolute changes in load and repetition progression were not different, there were relative differences such that the BFR training program had a greater percent increase in training load throughout the 12-weeks of training. This provides evidence that perhaps the initial KF training load (30% of 1-RM) was too low in the BFR training group. Investigating the effects of BFR exercise on KF strength and CSA should be further explored and KF exercise protocols should be evaluated to consider the rates of progression and manipulation of loads and repetitions.

The strengths of this study include the high compliance rates and the comprehensive assessment of resistance training progression in older adults classified as being at risk of mobility limitations. Despite not actually possessing mobility limitations but having a lot of variability in strength levels, the participants in our study benefitted from HL and BFR resistance training by gaining muscle mass, and improving muscle strength. Unfortunately, we did not conduct tests of muscular endurance and power and therefore we cannot make conclusions to determine the effect of the training interventions on those variables. It is suspected that the faster rate of progression in repetitions performed by the BFR group may lead to superior improvements in muscular endurance due to the principle of specificity. Enhanced muscular power leads to gains in physical function (Reid et al., 2015) and should be evaluated following BFR exercise interventions.

CONCLUSION

HL and BFR resistance training increase muscle mass and strength in the KE and KF muscle groups. HL training leads to more robust and favorable strength adaptations in the KE 10-RM and it may be attributed to the greater load and/or faster rate of progression of the load throughout the 12-week training period and the specificity of the testing modality. Future research should be aimed at optimizing BFR protocols for systematic load

progression throughout the entire training period for maximal gains in strength.

ETHICS STATEMENT

This study was carried out in accordance with the Declaration of Helsinki as recommended by the University of New Hampshire Institutional Review Board. All participants provided written informed consent.

AUTHOR CONTRIBUTIONS

SC conceived and designed the study, collected the data, performed the analysis, and wrote the manuscript. CC performed the analysis and wrote the manuscript.

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The Effects of Restriction Pressures on the Acute Responses to Blood Flow Restriction Exercise

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Purpose: No current guidelines or recommendations exist informing the selection of restriction pressure during blood flow restriction exercise (BFRE). Moreover, the effects of specific relative restriction pressures on the acute muscle, metabolic and cardiopulmonary responses to BFRE are unclear. The purpose of this study was to characterize these acute responses at different levels of restriction pressure.

Methods: Participants (n = 10) completed rhythmic isometric knee extension exercise across five experimental trials in a balanced randomized order. Three were BFRE trials {B-40 [restriction pressure set to 40% LOP (total limb occlusion pressure)]; B-60 (60% LOP); and B-80 (80% LOP)} with a workload equivalent to 20% maximal voluntary force (MVC), one was non-BFRE at 20% MVC (LL) and one was non-BFRE at 80% MVC (HL). Measurements recorded were torque, muscle activity via electromyography (EMG), tissue oxygenation via near infrared spectroscopy, whole body oxygen consumption, blood lactate and heart rate.

Results: For the LL and B-40 trials, most measures remained constant. However, for the B-60 and B-80 trials, significant fatigue was demonstrated by a reduction in MVC torque across the trial ($p < 0.05$). Blood lactate increased from baseline in HL, B-60, and B-80 ($p < 0.05$). Submaximal EMG was greater in B-60 and B-80 than LL, but lower compared with HL ($p < 0.05$). Tissue oxygenation decreased in HL, B-40, B-60, and B-80 ($p < 0.05$), which was lower in the B-80 trial compared to all other trials ($p < 0.01$). Whole body oxygen consumption was not different between the BFRE trials ($p > 0.05$).

Conclusion: We demonstrate graded/progressive acute responses with increasing applied pressure during BFRE, from which we speculate that an effective minimum "threshold" around 60% LOP may be necessary for BFRE to be effective with training. While these data provide some insight on the possible mechanisms by which BFRE develops skeletal muscle size and strength when undertaken chronically across a training program, the outcomes of chronic training programs using different levels of applied restriction pressures remain to be tested. Overall, the present study recommends 60–80% LOP as a suitable "minimum" BFRE pressure.

Keywords: Kaatsu, blood flow restriction, muscle fatigue, EMG, limb occlusion pressure, restriction pressure

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INTRODUCTION

To increase skeletal muscle size and strength it is recommended to lift loads that exceed 65–70% one repetition maximum (1-RM) (American College of Sports Medicine, 2009). However, exercise training using relatively light intensities [20–30% of maximal voluntary contraction (MVC)] in combination with an externally applied blood flow restriction (BFR) can elicit similar gains in skeletal muscle size and strength compared with traditional heavy-load resistance exercise (HLRE) (Shinohara et al., 1997; Karabulut et al., 2010; Yasuda et al., 2011). This light-intensity blood flow restriction exercise (BFRE) has significant practical application for a range of population groups that may be contraindicated to perform HLRE, such as older adults (Abe et al., 2010; Karabulut et al., 2010), athletes and/or patients recovering from musculoskeletal conditions such as anterior cruciate ligament injuries (Takarada et al., 2000b; Ohta et al., 2003). However, the restriction pressures applied during BFRE have historically been arbitrarily selected, and most often based on pressures used previously (Fahs et al., 2011; Takada et al., 2011; Hunt et al., 2013; Neto et al., 2016). This is independent of whether an absolute pressure is selected (Fahs et al., 2011; Hunt et al., 2013; Karabulut et al., 2013), or whether the pressure selected is individualized to structural and physiological characteristics of participants (Cook et al., 2007; Laurentino et al., 2012; Loenneke et al., 2013). Consequently, it so happens that few studies have attempted to examine the influence of different magnitudes of restriction pressure on acute responses to BFRE (Yasuda et al., 2008; Fatela et al., 2016), that may in turn influence the magnitude of any gains in skeletal muscle size and strength with chronic BFRE training.

A demonstrated acute response to BFRE is the greater rate of acute fatigue development than equivalent intensity light-load resistance exercise (LLRE) (Wernbom et al., 2008; Farup et al., 2015; Yasuda et al., 2015). However, until recently (Fatela et al., 2016), this has only been examined using single arbitrary pressures (Wernbom et al., 2008; Manimmanakorn et al., 2013; Farup et al., 2015). This heightened fatigue aligns with demonstrations of elevated skeletal muscle electromyography (EMG) activity across a BFRE bout (Wernbom et al., 2008; Yasuda et al., 2008, 2013; Fatela et al., 2016), leading many to speculate that this fatigue is compensated by increased muscle activation (Takarada et al., 2000c; Yasuda et al., 2008; Fatela et al., 2016). This elevated EMG activity is suggested to arise from increased Type II muscle fiber recruitment with BFRE (Takarada et al., 2000c; Fatela et al., 2016), and as such it has been speculated that Type II fibers are the primary source of the overall increased strength and muscle growth following chronic BFRE training (Loenneke et al., 2014; Pearson and Hussain, 2015). However, this has recently been challenged following 6.5 weeks BFRE training in powerlifters that showed greater proliferation of myonuclei and expansion of fiber area in Type I compared with Type II muscle fibers (Bjørnsen et al., 2018), although again this study used only a single arbitrary pressure.

The proposed greater type II fiber recruitment in response to BFRE has previously been reinforced by a greater accumulation of blood lactate (BLa) (Takarada et al., 2000c; Takano et al., 2005; Kim et al., 2014; Staunton et al., 2015), which inherently arises when Type II fibers are active (Pearson and Hussain, 2015). This suggests greater muscle acidity during BFRE compared with LLRE that likely stimulates growth hormone (GH) release (Takarada et al., 2000a; Takano et al., 2005; Fujita et al., 2007), which may induce subsequent muscle hypertrophy contributing to the increase in strength with chronic training (Takarada et al., 2000c; Abe et al., 2006). However, the BLa response to BFRE under different levels of applied restriction pressure remains untested. Similarly, the metabolic effect of BFRE is assumed to be associated with the level of hypoxia within the downstream musculature and other tissues (Moritani et al., 1992; Gentil et al., 2006). While hypoxia may be associated with mechanisms of muscle growth through effects on muscle fiber recruitment (Pearson and Hussain, 2015) or tissue growth factors [e.g., hypoxia-inducible factor-1α (HIF-1α)] (Taylor et al., 2016), the measurement of factors associated with hypoxia (e.g., muscle tissue oxygen saturation) remain unclear. Despite one investigation of tissue oxygen saturation across different levels of applied restriction pressure (Downs et al., 2014), this was examined with exercise to failure. As such, it seems pertinent to further examine the metabolic and hypoxic response to BFRE at different levels of applied restriction pressure.

Therefore, the aims of this study were to examine the acute BFRE responses of muscle fatigue, muscle activation, metabolism (BLa, hypoxia via tissue oxygenation) and whole-body cardiopulmonary responses to BFRE with different levels of applied restriction pressure, while also comparing against LLRE and HLRE. We anticipate that an understanding of these acute responses will assist to inform cuff pressure selection for future research that examines chronic BFR training regimens designed to develop muscle strength and size.

MATERIALS AND METHODS

Participants

Ten males (mean ± SD; 25 ± 6 years; 176.8 ± 5.6 cm; 78.14 ± 8.55 kg) were recruited to participate in this study. All were non-smokers who had not participated in any resistance exercise in the past 6 months and were currently undertaking no more than 150 min of physical activity per week. Relatively sedentary participants were selected to minimize variability in baseline MVC torque. In addition, we targeted sedentary participants given our group's view that BFRE is more relevant and applicable to sedentary cohorts, whether healthy or clinically affected. Of note, we also did not recruit women to reduce the possible variability in cardiovascular function in response to the menstrual cycle. Future research should examine both acute and chronic responses in different sexes. Prior to commencement in the study, participants completed a pre-screening questionnaire and provided written informed consent. Exclusion occurred for any persons with cardiovascular or musculoskeletal conditions or anyone taking medications for cardiovascular or blood pressure control. Participants attended the laboratory at the same time of day for each trial to avoid diurnal influences and were asked to refrain from exercise, and caffeine or alcohol consumption

in the 24 h before trials. All subjects provided written informed consent in accordance with the Declaration of Helsinki. The Deakin University Human Ethics and Advisory Group approved this study (HEAG-H 08_2017).

Study Design

Each participant attended the laboratory on six separate occasions. After an initial familiarization session, five experimental trials were conducted in a balanced, randomized crossover design, in which trials were performed in a random order on separate days, with at least 3 days between trials. The five experimental trials were: a heavy-load trial (80% MVC) without a restriction to blood flow (HL); a low-load trial (20% MVC) without a restriction to blood flow (LL) and; three low-load trials (20% MVC) performed at 40% (B-40), 60% (B-60), and 80% (B-80) of pre-exercise resting limb occlusion pressure (LOP).

Familiarization

Familiarization served the purpose of determining baseline characteristics for several measurements [muscle thickness (MTH), fat thickness (FTH), maximal whole body oxygen consumption ($\dot{V}O_2$max), and MVC] and included standard anthropometric measures [height, body mass, blood pressure (BP), and limb circumference]. MTH and FTH were measured over the *Vastus Lateralis* (*VL*) via 2D ultrasound. A linear array transducer (L12-5) interfaced with an ultrasound system (iU22; Philips Medical Systems, Sydney, NSW, Australia) was placed two-thirds of the way down the line from the anterior superior iliac spine to the lateral side of the patella. MTH and FTH were then measured using on-line calipers in triplicate. Participants then performed a standard incremental cycling test to exhaustion on a cycle ergometer (Lode Excalibur Sport, Lode, Groningen, Netherlands). Expired gases were collected throughout to determine $\dot{V}O_2$max. Participants respired through a mouthpiece with nose clip attached, into a standard metabolic cart (Innocor, Innovision A/S, Odense, Denmark). The protocol started at 50 W and then increased by 50 W every 3 min until 9 min, after which the workload increased by 25 W each minute until exhaustion. $\dot{V}O_2$max was defined as the highest whole body oxygen consumption ($\dot{V}O_2$) recorded over over a 15-s period during the test. Following a 10-min rest period, participants were seated on an isokinetic dynamometer (Biodex system 4 pro, Biodex Medical Systems, Shirly, United States). Participants were then instructed to perform three unilateral isometric knee extensions at maximal intensity using the dominant leg, with a 1-min rest between each contraction in order to familiarize them with the measurement of maximal voluntary contractile force. Finally, participants completed a short practice of the B-40 trial, performing the first set only to familiarize participants with BFRE and performing each rhythmic isometric contraction at the fixed cadence maintained during the experimental trials (3-s contraction and 1-s relaxation).

Experimental Trials

All five experimental trials were conducted in a random order, on separate days. Prior to commencement of each trial, participants completed a 5-min warm-up on a cycle ergometer at 75 W. Participants were then seated in an upright position on an isokinetic dynamometer. After all measurement devices were prepared, a total of 3 unilateral isometric knee extensions at maximal intensity were performed using the dominant leg. The greatest MVC was used to determine the load used during the subsequent exercise. The trial began 5 min after the completion of the MVC's. In all trials, participants performed supervised unilateral rhythmic isometric knee extensions. Repetitions were performed at a fixed cadence (3-s contraction, 1-s relaxation) guided by visual prompts on the Biodex screen, informing participants when to contract and relax, as well as the intensity required to be maintained for each repetition. In each trial, the first and final repetition of each set required participants to perform a MVC. For the LL trial and all BFRE trials, an initial set of 30 repetitions, followed by 3 sets of 15 repetitions were performed with a 30-s rest between each set (**Figure 1**). For the HL trial, 4 sets of 8 repetitions were performed with 2.5 min rest between each set (**Figure 1**). Isometric exercise was chosen given the greater suitability for measurement of tissue oxygenation and fatigue during the experimental trials. In combination with the selected duty cycle and target intensity (20%) it was expected that this would not overwhelm any distinguishable effect of the different applied restriction pressures on the variables used to characterize the acute response to BFRE.

Blood Flow Restriction Trials

In all BFRE trials, participants were fitted with an inflatable pneumatic cuff (86 cm long, 10.5 cm wide: bladder width 8 cm) applied to the most proximal portion of the dominant thigh. The cuff was connected to an automated tourniquet system (A.T.S. 3000, Zimmer, OH, United States) which was used to determine LOP at rest, as previously described (Staunton et al., 2015). LOP was then used to set and adjust the pressure during each BFRE trial. The cuff was inflated to the desired pressure over a 20-s period (40, 60, or 80% LOP) and 10 s later the trial commenced. Pressure was applied continuously throughout each of the sets and during rest periods until the completion of exercise, at which point the cuff was immediately deflated (8 min inflation per BFRE trial).

Measurements

Torque

Torque data was collected throughout the protocol and transmitted as voltage to be recorded in Powerlab 8 (Powerlab 16/35, ADInstruments, Australia). A monitor connected to the Biodex provided participants with visual feedback of the required level of torque when performing contractions. Additionally, verbal feedback/encouragement was given to ensure each participant consistently targeted the required submaximal load and reached maximum values during MVC's. Following the trial, the MVC torque data was normalized as a percentage of the highest MVC (raw torque) recorded pre-exercise for analysis.

Muscle Activity

Muscle activity in the *VL* was measured using EMG throughout the entire protocol. Electrode placement for the *VL* muscle occurred 2/3 of the way down the line from the anterior

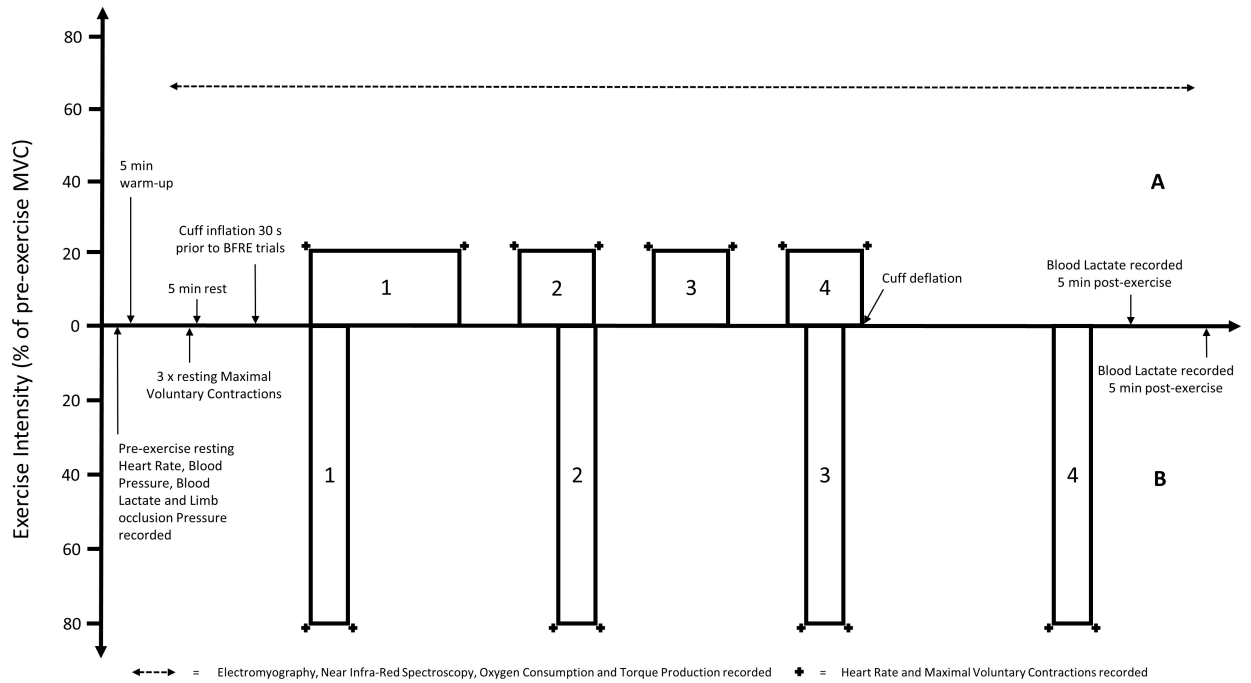

FIGURE 1 | Visual representation of the timing and intensity [based on pre-exercise maximal voluntary contraction (MVC)] of the **(A)** light load resistance exercise trials [including all blood flow restriction exercise (BFRE) trials] and **(B)** heavy load resistance exercise trial. Note that the pre- and post-exercise measurements are not to scale.

superior iliac spine to the lateral side of the patella (Hermans et al., 2000). A single ground electrode was fitted on the medial side of the tibial tuberosity. These sites were shaved, then scrubbed with an abrasive gel (Nuprep, Weaver and Company, United States) to remove hair and dead skin cells. The areas were then cleaned with a 70% isopropyl alcohol swab to ensure a clear signal was obtained. Bipolar (2-cm center-to-center) surface EMG electrodes (Ag-AgCl, Foam Electrodes, Covidien, Canada) were used to obtain EMG signals. An analog bandpass filter was applied at a sampling rate of 4000 Hz with a bandwidth of 13 – 1000 Hz. Powerlab was used through an analog-digital interface for analysis. A 500-ms root-mean-square (RMS) value was then analyzed from the plateau of each contraction to indicate muscle activity. The data was then normalized as a percentage of the EMG of the highest MVC (raw torque) recorded pre-exercise for analysis. Prior to analysis, the data were arranged to display both MVC EMG and the average EMG of the final 3 submaximal contractions of each set.

Tissue Oxygenation

The level of tissue oxygenation within the *VL* was measured throughout the entirety of each trial by near-infrared spectroscopy (NIRS) using a tissue oximeter sensor (NIRS: Portalite mini 40 mm × 20 mm × 5 mm, Artinis Medical Systems, Netherlands). The NIRS device was placed 2/3 of the way down the line from the greater trochanter to the lateral side of the patella and taped to the leg to ensure that the transmitter-receiver optode distance was always consistent during muscle contraction and/or movement. A black towel was placed over the NIRS device to screen any ambient light. Data was sampled throughout the protocol at a rate of 10 Hz and was recorded as concentration change from baseline in micromoles. A 500-ms average of the samples during each contraction and the immediately following 1 s rest period was recorded. The average of the final 10 s of each set and the average of the middle 15 s of each rest period were recorded for statistical analysis. The variable of interest used for analysis was the "tissue saturation index" (TSI), which is the ratio of signals representing oxy- and deoxy-hemoglobin.

Whole-Body Oxygen Consumption

Before each test the O_2 and carbon dioxide (CO_2) analysis systems were calibrated using ambient air and a gas of known O_2 and CO_2 concentration according to the manufacturer's instructions. The turbine flow-meter was calibrated using a 3-L syringe. Throughout the entirety of each trial, participants performed open-circuit spirometry, breathing into a mouthpiece with nose clip into a standard metabolic cart. Expired gas measures of $\dot{V}O_2$ were averaged into 15-s intervals for analysis.

Blood Lactate

Blood lactate was measured from a seated position at rest prior to the commencement of any exercise and then 5 min after the completion of the protocol (to allow for normal circulation of BLa post-deflation in the BFRE trials). BLa was taken via finger-prick on each participant's non-dominant hand and recorded on a lactate analyzer (Lactate Pro, Arkray Inc., Japan).

Heart Rate

Heart rate (HR) was first recorded after 5 min of seated rest. Subsequent HR recordings were taken immediately prior-to and following the first and last contraction of each set, respectively. HR was measured using a standard HR monitor watch receiver with chest strap transmitter (Polar, A300, Polar, NSW, Australia).

Blood Pressure

Brachial artery blood pressures [systolic (sBP) and diastolic (dBP)] were measured after 5 min of seated rest before the commencement of each trial. Measurements were taken in the upper arm via automatic auscultation using the non-invasive blood pressure feature on the Innocor system (Innocor, Innovision A/S, Odense, Denmark). Mean arterial pressure (MAP) was also calculated, using sBP, dBP and HR as inputs to determine this value [MAP = dBP + (0.01*EXP(4.14 – 40.74/HR)*(sBP-dBP))] (Moran et al., 1995).

Statistical Analysis

All data are presented as mean ± SEM, unless otherwise specified. MVC torque, MVC EMG, submaximal EMG, $\dot{V}O_2$ and TSI data were analyzed by a linear mixed model with repeated measures for fixed factors of Trial (HL, LL, B-40, B-60, and B-80) and Time (MVC torque and MVC EMG = Baseline, prior to, and following each set; submaximal EMG = set 1, 2, 3, and 4; $\dot{V}O_2$ and TSI = prior to and following each set) followed by pairwise comparisons. BLa and HR data were analyzed using repeated measures analyses of variance (ANOVA) for Trial and Time (BLa = pre- and post-exercise; HR = prior to, and following each set). Additionally, one-way ANOVA's were run to assess for differences at baseline in all measures between trials. For all analyses, significance was set at $p < 0.05$. All statistical analyses were performed using NCSS [NCSS 12 Statistical Software (2018), NCSS, LLC, Kaysville, Utah, United States].

TABLE 1 | Participant characteristics.

Age (years)	25 ± 6
Height (cm)	176.8 ± 5.6
Body mass (kg)	78.1 ± 8.6
BMI ($kg.m^{-2}$)	24.8 ± 1.6
Systolic blood pressure (mmHg)	114 ± 8
Diastolic blood pressure (mmHg)	66 ± 7
Mean arterial pressure (mmHg)	82 ± 6
Limb circumference (cm)	56.3 ± 3.5
Muscle thickness (cm)	3.47 ± 0.56
Thigh subcutaneous fat thickness (cm)	0.70 ± 0.18
$\dot{V}O_2$max ($l.min^{-1}$)	3.25 ± 0.44
$\dot{V}O_2$max ($ml.kg.min^{-1}$)	41.1 ± 6.8

Data is presented as Mean ± SD.

RESULTS

Baseline Characteristics

Participant baseline characteristics and anthropometric data align with the selection criteria, such that participants were relatively physically inactive and demonstrated average fitness ($\dot{V}O_2$max) (**Table 1**). Baseline MVC was not different between trials (**Table 2**). However, for the HL trial the target torque (80% MVC) achieved by participants was slightly below expected (71 ± 2% MVC) (**Table 2**), while still being classed as HLRE (American College of Sports Medicine, 2009). Resting LOP was not different between the BFRE trials (**Table 2**). Moreover, the applied restriction pressure during exercise was progressively greater from B-40 to B-60 to B-80 ($p < 0.05$).

MVC Torque

Torque recorded for each pre- and post-set MVC is shown in **Figure 2A**. There was a significant main effect for Time such that torque declined progressively across time from baseline. However, without a significant interaction (Time × Trial; $p = 0.08$) this decline was similar between trials, despite a significant main effect for Trial whereby B-80 was significantly lower than all other trials ($p < 0.001$), B-60 was significantly lower than LL and HL but not B-40 ($p = 0.052$), and B-40 was significantly lower than HL, but not LL. There was no difference between HL and LL ($p = 0.056$).

Muscle Activity

Maximal

Electromyography activity for each pre- and post-set MVC is shown in **Figure 2B**. There was a main effect for Time such that EMG activity significantly increased from pre- to post- for each set, but EMG activity also significantly declined from post-set to the subsequent pre-set. There was no significant interaction (Time × Trial). However, there were several main effects for Trial such that LL (90 ± 5% Baseline MVC EMG) was similar to all trials except B-80 (79 ± 8%), B-80 was also significantly lower than B-60

TABLE 2 | Baseline torque, target torque and restriction pressure for each trial.

Trial	Baseline MVC torque (N.m)	Target torque (N.m)	Achieved torque (% MVC)	LOP (mmHg)	Restriction pressure (mmHg)
LL	155.2 ± 13.5	31.0 ± 2.7	22 ± 1	–	–
HL	158.6 ± 10.4	126.9 ± 8.3*	71 ± 2*	–	–
B-40	150.6 ± 7.5	30.1 ± 1.5	21 ± 1	227 ± 5	91 ± 2*
B-60	139.4 ± 7.2	27.9 ± 1.4	22 ± 0	226 ± 9	136 ± 5*
B-80	155.0 ± 9.0	31.0 ± 1.8	20 ± 1	223 ± 7	178 ± 6*

*Data is presented as mean ± SEM. *Indicates a significant difference from all other trials ($p < 0.05$).*

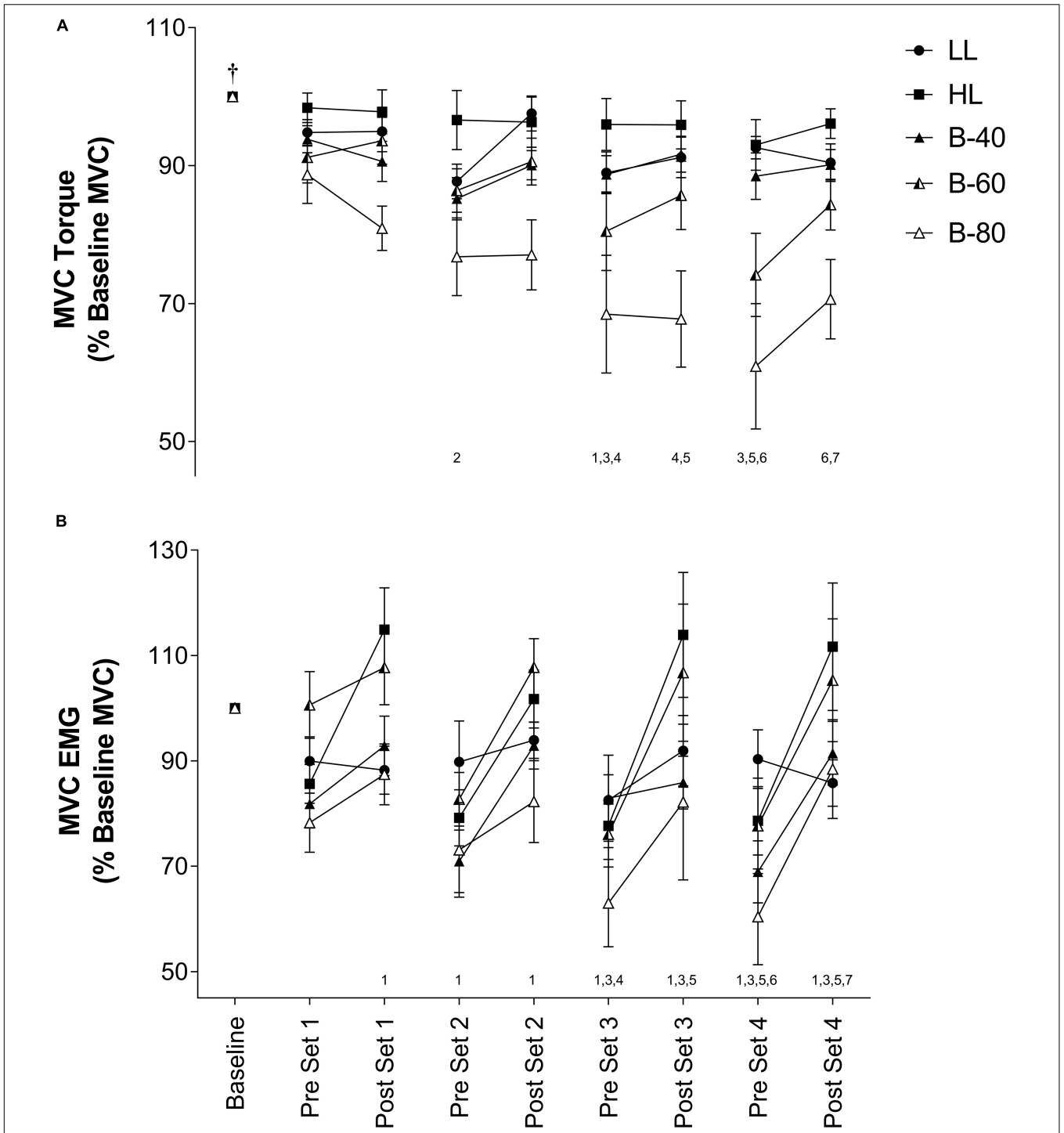

FIGURE 2 | (A) Maximal voluntary contraction (MVC) torque and **(B)** MVC electromyography (EMG) in the *Vastus Lateralis* muscle during all five trials [heavy load (HL), light load (LL), 40% limb occlusion pressure (B-40), 60% limb occlusion pressure (B-60), 80% limb occlusion pressure (B-80)]. Values are presented as mean ± SEM. Number values along *x*-axis represent a significant Time effect from the number of time points indicated prior. †Indicates a significant Time effect from all other time points. Significance set at $p < 0.05$.

(96 ± 7%) and HL (96 ± 6%), HL was significantly greater that B-40 (85 ± 5%) and B-40 was significantly lower than B-60 (96 ± 7%).

Submaximal

Average EMG activity over the last 3 submaximal contractions of each set is displayed in **Figure 3**. Submaximal EMG

activity remained constant over time (i.e., no main effect). However, there was a main effect for Trial such that submaximal EMG activity for HL was significantly higher than all other trials ($p < 0.001$), while both B-80 and B-60 were significantly greater than LL ($p < 0.01$). B-80 was also significantly greater than B-40 ($p < 0.01$), although B-60 was not ($p = 0.0508$). There was no significant interaction (Time × Trial).

Whole Body Oxygen Consumption

Mean whole body $\dot{V}O_2$ for the 15-s period pre- and post-set is displayed in **Figure 4A**. There was a main effect for Time, such that whole body $\dot{V}O_2$ increased across all trials ($p < 0.05$). There was also a main effect for Trial, such that submaximal $\dot{V}O_2$ was greater in HL than all other trials. A significant interaction (Time × Trial) showed $\dot{V}O_2$ for HL to be greater post-set than all other trials ($p < 0.001$), while for all trials, $\dot{V}O_2$ increased across all sets and declined during recovery between sets [except from post-set 1 and pre-set 2 for B-60 ($p = 0.07$)].

Muscle Oxygenation (TSI)

Mean TSI of *VL* for each 15-s period pre- and post-set is displayed in **Figure 4B**. There was a main effect for Time, and a significant interaction (Time × Trial) such that TSI decreased across the HL, B-40, B-60 and B-80 trials, while TSI remained unchanged across the LL trial. TSI declined significantly across set 1 for the HL, B-40, B-60, and B-80 trials ($p < 0.05$), without a change in the LL trial. While TSI recovered between sets for the HL trial only, TSI also declined across each of the remaining sets in HL only (2, 3, and 4). After declining across set 1 TSI remained unchanged across the three remaining sets for all BFRE trials (**Figure 4B**). However, in B-80 TSI declined significantly across the recovery period between set 1 and set 2. There was also a main effect for Trial such that TSI for B-80 was significantly lower than all other trials ($p < 0.001$), B-60 was significantly lower than LL, HL and B-40 ($p < 0.001$), and B-40 was significantly lower than HL ($p < 0.01$). In addition, the significant interaction showed TSI for B-80 to be lower than all trials from pre-set 2 onward.

Blood Lactate

Blood lactate concentration recorded pre- and post-exercise is displayed in **Figure 5A**. There was a significant main effect for Time and a significant interaction (Time × Trial) such that BLa concentration increased across the HL and B-80 trials, while remaining unchanged across the LL, B-40 and B-60 trials. The significant interaction also showed post-exercise BLa for both HL and B-80 to be greater than both the LL and B-40 trials ($p < 0.05$), while BLa was also significantly greater post-exercise in B-60 compared with LL.

Heart Rate

Heart rate recorded immediately pre- and post-set is shown in **Figure 5B**. There was a significant main effect for Time such that HR increased with exercise across each set and declined with recovery between sets. There was also a main effect for Trial such that HR for both HL and B-80 was greater than B-40 ($p < 0.05$). However, a significant interaction showed that post-exercise HR for HL was greater than LL and B-40 in all sets ($p < 0.05$), and greater for B-60 and

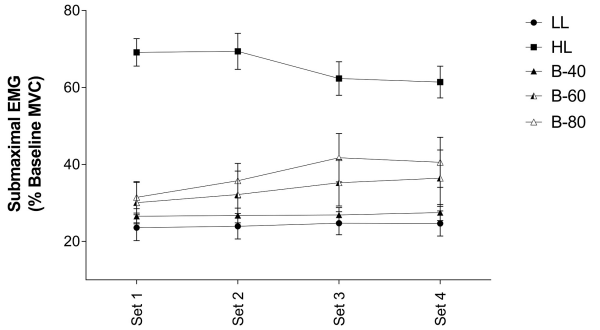

FIGURE 3 | Electromyography (EMG) of submaximal contractions in the *Vastus Lateralis* muscle during all five trials [heavy load (HL), light load (LL), 40% limb occlusion pressure (B-40), 60% limb occlusion pressure (B-60), 80% limb occlusion pressure (B-80)]. Values are presented as mean ± SEM. Significance set at $p < 0.05$.

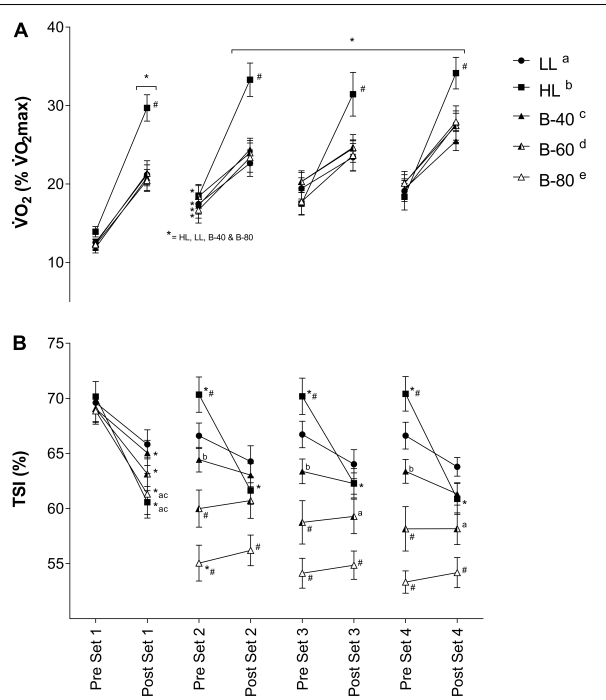

FIGURE 4 | (A) Whole body volume of oxygen consumption ($\dot{V}O_2$) and **(B)** Muscle oxygenation (TSI) of the *Vastus Lateralis* muscle during all five trials [heavy load (HL), light load (LL), 40% limb occlusion pressure (B-40), 60% limb occlusion pressure (B-60), 80% limb occlusion pressure (B-80)]. Values are presented as mean ± SEM. *Indicates a significant difference from previous time point. Lower case letters (see legend) represent a significant difference between trials at a time point. #Indicates a significant difference from all trials at a time point. Significance set at $p < 0.05$.

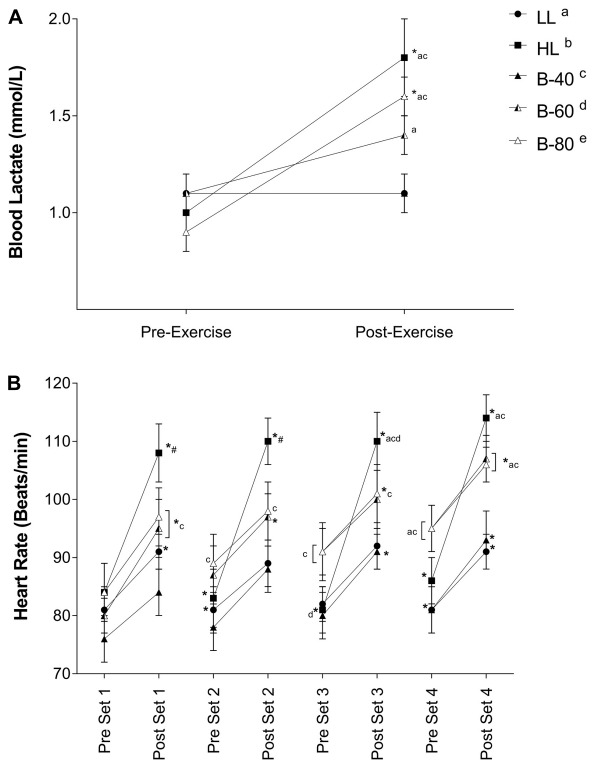

FIGURE 5 | (A) Blood lactate concentrations and **(B)** Heart rate during all five trials [heavy load (HL), light load (LL), 40% limb occlusion pressure (B-40), 60% limb occlusion pressure (B-60), 80% limb occlusion pressure (B-80)]. Values are presented as mean ± SEM. *Indicates a significant difference from previous time point. Lower case letters (see legend) represent a significant difference between trials at a time point. #Indicates a significant difference from all trials at a time point. Significance set at $p < 0.05$.

B-80 than LL and B-40 toward the end of exercise (e.g., pre-set 4 onward).

DISCUSSION

The present study investigated acute muscle (torque and EMG), metabolic (BLa and TSI) and cardiopulmonary (HR and $\dot{V}O_2$) responses to different levels of individualized applied restriction pressure during BFRE. The major finding was that, in general, increases to BFR pressure exaggerated the magnitude of the acute responses to BFRE. As the level of restriction pressure increased the torque and TSI response declined further, while the muscle activity and BLa response was amplified. The outcomes were similar between the B-60 and B-80 trials, and these trials were generally comparable to the HL trial, indicating that BFRE, with a cuff pressure of 60–80% LOP may be a suitable substitute where participants are contraindicated to perform HLRE. However, it is important to recognize that these outcomes are derived from the rhythmic isometric exercise of the present study, and while we expect this to be similar for dynamic exercise, it remains to be tested. In contrast, restriction pressure in the B-40 trial was not sufficient to produce acute responses beyond that of the LL trial.

Despite the target torque (20% MVC) being equivalent among the LL and BFR trials, muscle activity (*submaximal* EMG) required to produce these torques was positively associated with the magnitude of the applied BFR pressure such that muscle activity was lowest in the LL and B-40 trials and increased toward the B-60 then B-80 trials, and finally the HL trial (target torque = 80% MVC) (**Figure 3**). In contrast, when assessing fatigue across the trials by examining the decline in MVC torque where the B-60 and B-80 trials demonstrated greater fatigue when approaching completion of exercise (**Figure 2A**), the *maximal* EMG activity was lowest with the greatest restriction pressures (e.g., B-80; **Figure 2B**). Both these relationships between EMG and torque for *submaximal* and *maximal* responses are supported by previous findings, where increases in the applied restriction pressure led to a progressive increase in submaximal muscle activity and a decrement in maximal torque production (Yasuda et al., 2008; Fatela et al., 2016). The present study is limited by not measuring fatigue via supramaximal electric stimulation, and hence does not inform as to the origin of the observed fatigue, whether central or peripheral (Place et al., 2009). However, the measurement of MVC torque throughout the protocol (i.e., before and after each set) in addition to EMG, provides valuable information around the onset and progression of fatigue developed throughout a BFRE protocol.

Interestingly, there was a decline in MVC torque during the rest periods between some sets toward the end of exercise in both the B-60 and B-80 trials, which also occurred immediately upon cuff inflation prior to the commencement of exercise (**Figure 2A**). This acute progressive fatigue during the rest periods points substantially to the importance of continued restriction to flow between sets contributing to the potential mechanisms by which BFRE may generate increased muscle strength and size with training in combination with the contractile phase of the activity (Yasuda et al., 2013; Pearson and Hussain, 2015; Iversen et al., 2016). Moritani et al. (1992) previously speculated that deficits in force development may be caused by limited oxygen availability and other blood borne substrates (i.e., glucose and free fatty acids) and/or acidification of the intramuscular environment, with the recruitment of additional motor units serving to compensate for this effect. Therefore, the muscle fatigue, represented by a decrease in MVC torque, and associated increases to muscle activation within the B-60 and B-80 trials may be explained by changes to oxygen availability (TSI) and accumulation of metabolic by-products (lactate).

Muscle tissue oxygenation represented by TSI (**Figure 4B**) somewhat mirrors fatigue/MVC torque (**Figure 2A**). However, while fatigue appears progressive across the BFRE bout, in particular for B-80, the decline in TSI is initially rapid across set 1 and maintained from set 2 onward without any sign of recovery. This apparent persistent tissue hypoxia appears more severe with an increase in restriction pressure (B-60 to B-80) while being absent for B-40, so again indicates an effective BFR pressure being between 60 and 80% LOP. Of note, the HL bout also shows a significant and rapid decline in tissue oxygenation, but in this trial there is a rapid recovery of TSI to baseline

during rest periods between sets. Visually, this is inverse to $\dot{V}O_2$ for HL, and while the BFR and LL trials demonstrate a similar profile but with reduced magnitude for $\dot{V}O_2$ (**Figure 4A**), the lack of recovery of TSI between bouts in the B-60 and B-80 trials suggests that there is a disconnect between whole body $\dot{V}O_2$ and the level of tissue oxygenation with BFRE, at least with higher applied pressures that is not observed for HL. It is difficult to suggest whether this may shed light on the mechanism of muscle growth when BFRE is undertaken chronically across a training period. However, it provides evidence to support a hypothesis that BFRE and HL may stimulate muscle adaptation through divergent pathways.

The $\dot{V}O_2$ response shows an apparent metabolic demand of the exercise to be entirely dependent on the level of work. That is, HL generates the greatest metabolic demand, with all other trials generating similar but smaller demand independent of the level of BFR applied (i.e., B-40, B-60, and B-80) or not (LL). Although, this is in contrast with others that show BFR to increase metabolic requirements as assessed by $\dot{V}O_2$ (Eiken and Bjurstedt, 1987; Abe et al., 2006), where we used individualized pressures and resistance exercise, as opposed to aerobic walking (Abe et al., 2006) or cycling (Eiken and Bjurstedt, 1987) and particularly high pressures (200 mmHg, Abe et al., 2006), or particularly low pressures (50 mmHg, Eiken and Bjurstedt, 1987). However, when examining data for BLa there is a clear relationship between exercising load and the level of glycolytic metabolism that is highest for HL and lowest for LL. We show this to be modulated by the addition of BFR while also being sensitive to the level of the applied restriction such that there is no effect on BLa in the B-40 trial, but a progressive increase in BLa with increasing applied pressure to B-60 and then B-80 (**Figure 5A**). This aligns with the greater *submaximal* EMG activity with BFR and taken together this lends support for greater Type II fiber recruitment during BFRE (Pearson and Hussain, 2015). To some extent the BLa response also reflects HR (as opposed to $\dot{V}O_2$) whereby HL produces the highest HR's across the trial (again in line with the exercising load) while LL produces the lowest HR's (**Figure 5B**). B-40 again shows no evidence of an effect of BFR while B-60 and B-80 increase HR to a similar level to HL by the end of the trial. This response appears to be less graded (i.e., progressively related to the level of applied restriction pressure) but reinforces the suggestion that BFR impacts limb blood flow both during the active exercise periods and importantly during periods of rest between sets. In this respect the present study is limited by not having a direct measure of limb blood flow and, therefore, it is important that this suggestion be confirmed in future research. While we did not measure blood flow *per se* in the present study, our group and others have previously shown the generation of cardiac output for BFRE to be derived from higher HR's and lower stroke volumes (Brandner et al., 2015; Staunton et al., 2015; May et al., 2017), but the present study adds to this HR data by providing greater resolution to this response by including both rest and exercising HR's.

Limitations

One limitation of the present study is that without an objective supramaximal stimulation protocol applied during MVC's to assess fatigue, we cannot identify the origin of fatigue (central or peripheral) (Place et al., 2009), nor assess the level of volition from participants. However, we did not experience any variation in pre-exercise MVC torque between trials and therefore expect that participant effort was maximal for the pre-exercise measurement. Furthermore, the measurement of both MVC torque and EMG provides some valuable insight into the onset and progression of fatigue throughout a BFRE protocol. BFR may also have an effect on volitional effort during MVC's throughout exercise and as such it is important to test maximal contractions via supramaximal electrical stimulations in the future to obtain a more objective measure of fatigue. In addition, the present study measured rhythmic isometric contractions which limits extrapolation of the outcomes to more common BFR exercise training regimens. Although as outlined above, given the nature of the responses (i.e., most change occurred during rest), we do not expect the outcomes for more common dynamic BFR exercise to be vastly different.

CONCLUSION

The present study characterized the acute muscle (torque and EMG), metabolic (BLa and TSI) and cardiopulmonary (HR and $\dot{V}O_2$) responses to different levels of individualized applied restriction pressure during BFRE. While the findings of the present study should not be directly extrapolated to common dynamic modes of BFR exercise, given the graded response to BFR pressures and the nature of the decline in MVC torque, MVC EMG and tissue oxygenation during rest periods, we consider the outcomes of such modes would not be vastly different. We demonstrate these responses to be graded/progressive with increasing applied pressure, from which we speculate that an effective minimum "threshold" of 60% LOP to be necessary for BFRE to be potentially effective with training. In particular we show BFRE at, and above, this 60% LOP threshold to exacerbate fatigue and the metabolic stress of the exercise, while modulating the muscle activity required to complete the work requirements. While these data enable speculation on the possible mechanisms by which BFRE develops skeletal muscle size and strength when undertaken chronically across a training program, such as the importance of the impact of BFR on the rest periods between sets (and possibly between contractions), the outcomes of chronic training regimens using different levels of applied restriction pressures remain to be tested. Overall, notwithstanding the mode of exercise used in the present study, our findings recommend 60–80% LOP as a suitable BFR pressure.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Deakin University Human Ethics Advisory Group. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The

protocol was approved by the Deakin University Human Ethics Advisory Group (HEAG-H 08_2017).

AUTHOR CONTRIBUTIONS

MI, TR, and SW conceived and designed the study. MI, TR, and MK conducted the data collection. MI, SW, AM, and TR conducted the data analysis. MI, SW, AM, TR, and MK contributed to the writing of the manuscript.

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SYSTEMATIC REVIEW
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Chronic Blood Flow Restriction Exercise Improves Objective Physical Function: A Systematic Review

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Background: Blood flow restriction or KAATSU exercise training is associated with greater muscle mass and strength increases than non-blood flow restriction equivalent exercise. Blood flow restriction exercise has been proposed as a possible alternative to more physically demanding exercise prescriptions (such as high-load/high-intensity resistance training) in a range of clinical and chronic disease populations. While the maintenance of muscle mass and size with reduced musculoskeletal tissue loading appeals in many of these physically impaired populations, there remains a disconnect between some of the desired clinical measures for chronic disease populations and those commonly measured in the literature examining blood flow restriction exercise. While strength does play a vital role in physical function, task-specific objective measures of physical function indicative of activities of daily living are often more clinically relevant and applicable for evaluating the success of medical and surgical interventions or monitoring age- and disease-related physical decline.

Objective: To determine whether exercise interventions utilizing blood flow restriction are able to improve objective measures of physical function indicative of activities of daily living.

Methods: A systematic search of Medline, Embase, CINAHL, SPORTDiscus, and Springer identified 13 randomized control trials utilizing an exercise intervention combined with blood flow restriction, while measuring at least one objective measure of physical function. Participants were ≥18 years of age. Systematic review of the literature and quality assessment of the included studies used the Cochrane Collaboration's tool for assessing risk bias.

Results: Data from 13 studies with a total of 332 participants showed blood flow restriction exercise, regardless of modality, most notably increased performance on the 30 s sit-to-stand and timed up and go tests, and generally improved physical function on other tests including walking tests, variations of sit-to-stand tests, and balance, jumping, and stepping tests.

Conclusions: From the evidence available, blood flow restriction exercise of multiple modalities improved objective measures of physical function indicative of activities of daily living.

Keywords: blood flow restriction, KAATSU, physical function, exercise, training, activities of daily living

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INTRODUCTION

Strength or resistance training is a primary exercise modality in exercise prescription guidelines for healthy adults (American College of Sports Medicine, 2009), older adults (Nelson et al., 2007), and many clinical populations (Moore et al., 2016). Maintaining or improving muscle mass and strength is imperative for not only higher-level sports performance, but essential musculoskeletal function, which includes common tasks like ambulation, balance, and activities of daily living (ADL) (Garber et al., 2011). Traditionally, maintaining muscle mass and strength with high-load resistance training (HLRT) utilizes loads >70% of an individual's one-repetition maximum (1RM) (American College of Sports Medicine, 2009). However, HLRT is often perceived as being too difficult or technique-intensive for novices (Thiebaud et al., 2014a), or may be contraindicated for certain populations, such as frail individuals, people living with chronic disease, or those in early stage musculoskeletal rehabilitation (Williams et al., 2007; Vanwye et al., 2017). In recent years, low-intensity exercise (20–30% 1RM) combined with blood flow restriction (BFR) has been proposed as a viable alternative to HLRT for maintaining or improving muscle mass and strength (Lixandrao et al., 2018).

A recent systematic review and meta-analysis concluded that HLRT remains a more practical option than low-intensity resistance training with BFR (BFR-RT) for improving strength among individuals able to perform HLRT (Lixandrao et al., 2018). However, development of muscle mass was deemed equally effective with either HLRT or BFR-RT (Lixandrao et al., 2018). Additionally, low-to-moderate intensity aerobic exercise training combined with BFR (BFR-AT) has also been shown to increase muscle mass and strength beyond its non-BFR equivalent (Slysz et al., 2016). While BFR-AT is likely less effective for increasing muscle mass and strength compared with HLRT or BFR-RT, it requires notably less mechanical, haemodynamic and perceptual stress than either HLRT or BFR-RT (May et al., 2017; Neto et al., 2017a; Vanwye et al., 2017). Collectively, BFR-RT and BFR-AT both cater to a broad spectrum of physical abilities among different populations, who may be contraindicated or otherwise opposed to HLRT as a means of maintaining or improving muscle mass and strength.

Both BFR-RT and BFR-AT have been proposed as possible alternatives to more physically demanding exercise prescriptions in a range of clinical and chronic disease populations such as chronic obstructive pulmonary disorder (Thiebaud et al., 2014b), end-stage kidney disease (Clarkson et al., 2017a), ischemic heart disease (Madarame et al., 2013), and inclusion body myositis (Jorgensen et al., 2018). While the ability to maintain muscle mass and size with a reduction in musculoskeletal tissue loading appeals in many of these physically impaired populations, it has not translated into larger scale randomized controlled trials among these populations. Some studies provide proof of concept pilot data among chronic disease populations (McCully et al., 2004; Madarame et al., 2013; Mattar et al., 2014; Jorgensen et al., 2018) or outline the relative haemodynamic safety of the technique (Jessee et al., 2017; May et al., 2017; Neto et al., 2017b; Barili et al., 2018). However, there remains a disconnect between some of the desired clinical measures for chronic disease populations and those commonly measured in the BFR literature. While strength does play a vital role in physical function (Buchner et al., 1997), task-specific objective measures of physical function that are indicative of ADL are often more clinically relevant and applicable for evaluating the success of medical and surgical interventions, or monitoring age- and disease-related physical decline (Groll et al., 2005). As such, the term "physical function" in the present review refers specifically to the ability to independently perform activities of daily living.

Previous systematic reviews of BFR exercise modalities have almost exclusively measured muscle mass and strength with regard to physical outcomes (Loenneke et al., 2012; Slysz et al., 2016; Lixandrao et al., 2018). One review did include mention of the importance of physical function but specifically for musculoskeletal rehabilitation, although this was not a primary focus of the review and instead highlighted the lack of investigation into the examination of physical function with BFR exercise training (Hughes et al., 2017). Other reviews have explored the effects of BFR exercise on bone metabolism (Bittar et al., 2018), haemodynamic responses to BFR exercise (Neto et al., 2017a), or the mechanisms and relative safety of the technique (Fahs et al., 2012; Patterson et al., 2017). Therefore, the purpose of this systematic review was to elucidate the efficacy of both BFR-RT and BFR-AT for improving a range of measures of objective physical function indicative of ADL.

METHODS

Study Design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Search Strategy

The electronic database search included Medline, Embase, CINAHL, Springer, and SPORTDiscus. Search strategy utilized the search strings identified in the **Supplementary Material**. Search terms were derived from "physical function," "blood flow restriction," and "exercise." References were also identified in the reference lists of previous systematic reviews in addition to the results of our electronic database search. Search results were filtered within the database where possible for the filters "Human," "English," "randomized controlled trial," "controlled trial," "clinical trial," "controlled clinical trial," "journal," "journal article," and/or "academic journal". Search results included dates from inception until the date of the search (25th November 2018).

Participants, Interventions, Comparators

Database search results were imported into Endnote X8 (Thompson Reuters, Philadelphia, Pennsylvania, USA). Duplicates were removed, and screening was completed by title, abstract, and full text. Excluded articles were sorted into individual folders indicating the reason for exclusion until only articles for inclusion remained. This process was completed by two researchers independently. The relevant inclusion criteria

are identified below and reasons for exclusions noted in the PRISMA flow chart (**Figure 1**):

1. Language: only studies published in English were included in this review.
2. Study Design: only studies that employed a randomized control trial (RCT) design were included. Systematic reviews, narrative reviews, conference abstracts, editorials, letters or publications not-inclusive of original data were excluded.
3. Intervention: studies must have included an exercise training intervention in the form of chronic aerobic, resistance, combined, or alternative types of progressive exercise training or significant chronic muscular activation in the primary intervention group or groups over multiple weeks. The primary intervention group must have used BFR during the prescribed exercise training.
4. Controls: control groups in these studies must have been non-BFR equivalent exercise, non-exercising controls, or alternative traditional exercise prescriptions. Within participant controls (single limb interventions) were excluded from the review.
5. Outcomes: must have included at least one objective measure of physical function indicative of ADL. Subjective measures associated with physical function (questionnaires or surveys) were excluded.

Examples of objective measures of physical function indicative of ADL include the 6-min walk test (6MWT), variations of the sit-to-stand test, balance tests, or grip strength tests, which have similarities in their execution to everyday activities. Measures excluded from this review include laboratory tests such as maximal strength testing, or graded exercise testing utilizing measures of oxygen utilization, ventilatory or lactate threshold, as these are not reflective of ADL.

Assessment of Risk Bias

The risk of bias of included studies was independently evaluated by two reviewers (MJC, AKM) using the Cochrane Collaboration's tool for assessing risk bias (Higgins and Green, 2011). The overall quality assessment of the RCTs included analysis of both selection bias, detection bias, and attrition bias. Selection bias was examined through method of recruitment, protocol for randomization, concealment of treatment allocation, and similarity of groups' baseline characteristics. Detection bias included blinding of assessors to intervention groups and possible blinding of participants. Attrition bias explored level of adherence of participants, completeness of follow up, and reported reasons for attrition. Contention between quality assessments was resolved through follow up consultation between reviewers. Each component of the bias assessment was assigned a rating of high, low, or unclear risk of bias, sufficient enough to notably impact results or the conclusions of the trial.

Data Extraction

Following the initial screening, information from identified studies that was extracted included basic study characteristics, mean participant age, sample size, control group intervention modality, and duration, experimental intervention modality and duration, and measures of objective physical function.

RESULTS

Literature Search

In total, 4,563 articles were retrieved from searches from inception to 25th November 2018 from Medline (267), Embase (2,154), CINAHL (795), Springer (691), and SPORT Discus (656). Duplicates were removed to refine the total number of articles for screening down to 3,783. Of these 3,783 articles screened for eligibility, 3,450 were excluded based on title or abstract. The full texts of the remaining 333 articles were evaluated based on the inclusion criteria for this review. Of these, 169 were excluded by study design for not being a RCT or not being a training study, 151 were excluded for not measuring an objective measure of physical function indicative of ADL, and 1 full text was unable to be obtained. Ultimately, 12 studies fulfilled the criteria and were included in the current review. An additional article was identified from the reference lists of prior reviews in the field identified as part of the search and was added to the analyses for a total of 13 included studies.

Study Selection and Characteristics

The studies included in this review are summarized in **Table 1** based on sample size, population, exercise modality and duration for both the BFR and control groups, outcome measures, and main findings. The 13 studies included a total of 332 participants. Individual studies generally consisted of small sample sizes, ranging from $n = 17$ (Tennent et al., 2017) to $n = 48$ (Ferraz et al., 2018), with only six studies examining more than the mean number of participants for all studies (26 participants) (Araujo et al., 2015; Bryk et al., 2016; Cook et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Ladlow et al., 2018). Among all studies there was a relatively even spread of participants between the BFR intervention groups (mean $n = 12$) and the comparison groups (mean $n = 12$). The populations examined among studies exploring physical function after BFR exercise training were variable. However, the most commonly examined population was older adults (aged 60 years or over) which was examined in six of the included studies (Yokokawa et al., 2008; Abe et al., 2010; Ozaki et al., 2011; Yasuda et al., 2014; Clarkson et al., 2017b; Cook et al., 2017). Other populations examined were generally healthy adults (Ladlow et al., 2018), women with knee osteoarthritis (Bryk et al., 2016; Ferraz et al., 2018), post-menopausal women (Araujo et al., 2015), adults post-arthroscopic knee surgery (Tennent et al., 2017), patients with end-stage kidney disease (Barbosa et al., 2018), and patients with sporadic inclusion body myositis (Jorgensen et al., 2018). The majority of studies employed a control group completing non-BFR equivalent exercise (Ozaki et al., 2011; Araujo et al., 2015; Bryk et al., 2016; Clarkson et al., 2017b; Tennent et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018), or an inactive comparison group (Abe et al., 2010; Yasuda et al., 2014; Cook et al., 2017; Jorgensen et al., 2018). However, three of these studies had a second comparison group; one study utilized an inactive control as well as their non-BFR equivalent exercise group (Araujo et al., 2015), and two studies

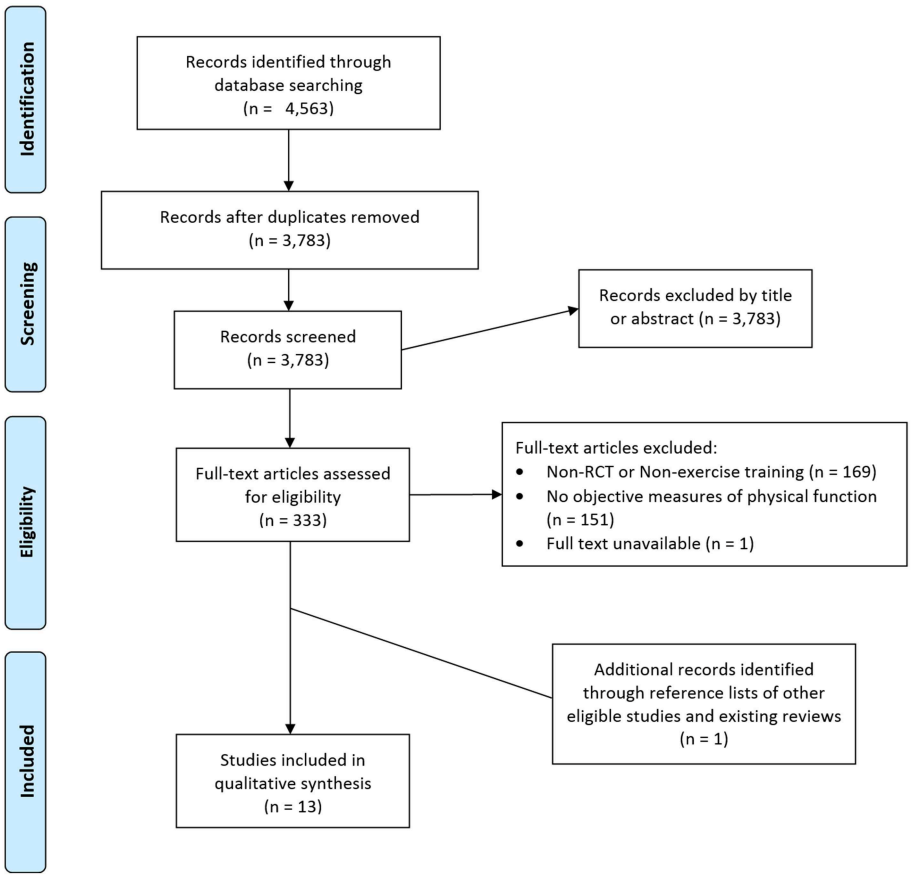

FIGURE 1 | PRISMA flow chart of study selection process.

included a HLRT comparison group in addition to a non-BFR equivalent exercise group (Ferraz et al., 2018), or an inactive control group (Cook et al., 2017). One other study used HLRT as their only comparison group (Ladlow et al., 2018). One study used fundamental balance exercises as an intervention for their comparison group, as this was the common practice exercise prescription for improving the outcome measures assessed in the study, and thus a suitable comparison to the novel use of BFR-RT in their primary intervention group (Yokokawa et al., 2008). Only five studies attempted to report adverse events among participants (Yokokawa et al., 2008; Cook et al., 2017; Tennent et al., 2017; Ferraz et al., 2018; Ladlow et al., 2018). Four of these studies did not have any adverse events (Yokokawa et al., 2008; Cook et al., 2017; Tennent et al., 2017; Ladlow et al., 2018), and one study reported four cases of involved exercise-induced knee pain leading to discontinuation in the study, all of which occurred following HLRT alone (Ferraz et al., 2018).

Risk of Bias Assessment

Selection Bias

One inclusion criteria for this review was that studies had to be randomized controlled trials. Therefore, most studies had adequate randomization or participant allocation (Ozaki et al., 2011; Araujo et al., 2015; Bryk et al., 2016; Clarkson et al., 2017b; Cook et al., 2017; Tennent et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018). Concealment of the randomization method was adequately described in only five of the included studies (Bryk et al., 2016; Clarkson et al., 2017b; Tennent et al., 2017; Barbosa et al., 2018; Ladlow et al., 2018).

Detection Bias

The process used to blind participants and study personnel was adequately described in only a single study, in which the BFR cuffs were also applied to participants in the non-BFR exercise group without inflation as a method of blinding (Barbosa et al., 2018). Collectively, only five studies used blinded assessors for the outcome assessments (Bryk et al., 2016; Tennent et al., 2017; Barbosa et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018), while one other study displayed a high risk of detection bias due to the outcome assessor being the same researcher who completed all training sessions and statistical analyses in the study (Clarkson et al., 2017b).

Attrition Bias

Nine of the thirteen included studies reported attrition and compliance of participants (Yokokawa et al., 2008; Bryk et al.,

TABLE 1 | Summary of studies evaluating changes in objective measures of physical function following exercise intervention combined with blood flow restriction.

References	Sample (Population, age)	Intervention details				Comparison group(s)		Physical function outcome (BFR vs. comparison)
		n	Duration	Modality	BFR prescription	*n*	Prescription	
Jorgensen et al. (2018)	Sporadic inclusion body myositis; 69 ± 6 years	11	12 weeks	Resistance training	10 cm cuff width, 110 mmHg pressure; 2 Sessions per week; 3–4 sets of 25 repetitions of leg press, knee extension, knee flexion, calf raise, dorsi-flexion	11	Inactive control group	↔ 2MWT ↔ 30STS ↔ TUG (no sig. Δ for any measure)
Barbosa et al. (2018)	End-stage kidney disease; ≥ 18 years	12	8 weeks	Resistance training	50% SBP using basic tensiometer; 2 Sessions per week; 6 sets of 10 tennis ball squeezes, 3 sets of 10 bicep curls (1–3 kg), 3 sets of 20 repetitious of 40% 1RM handgrip exercise	14	Non-BFR equivalent exercise group	↔ Handgrip strength (no sig. Δ)
Ladlow et al. (2018)	Healthy Adults; 31 ± 7 years	14	3 weeks	Resistance training	10 cm cuff width, 60% LOP (124 ± 13 mmHg); 9 Sessions per week; 4 sets as 30/15/15/15 repetitions at 30% 1RM for leg press and knee extension	14	HLRT: 4 sets of 6-8 repetitions for deadlift, back squats, lunges at 6RM	↑ Multi-stage locomotion test ↑ Y-Balance test
Ferraz et al. (2018)	Women with knee osteoarthritis; 60 ± 4 years	16	12 weeks	Resistance training	17.5 cm cuff width, 70% LOP (97 ± 8 mmHg); 2 Sessions per week; 4-5 sets of 15 repetitions of leg press and knee extension at 30% 1RM	1. 16. 2. 16.	1. Non-BFR equivalent exercise group 2. HLRT: 4 sets of 10 repetitions of leg press and knee extension at 80% 1RM	↔ 30STS ↔ TUG (no sig. Δ)
Tennent et al. (2017)	Post-operative knee arthroscope; 37 ± 17 years	10	6 weeks	Resistance training	80% LOP; 2 Sessions per week; 4 sets as 30/15/15/15 repetitions of leg press, leg extension, and leg curl at 30% 1RM (*in addition to traditional physical therapy*)	7	Traditional physical therapy for knee arthroscope (immediate weight bearing, immediate formal physical therapy, and unrestricted range of motion)	↔ STS5 ↔ 4SST ↔ Self-selected gait speed ↑ Timed stairs
Clarkson et al. (2017b)	Older adults; 70 ± 7 years	10	6 weeks	Aerobic training	10.5 cm cuff width, 60% LOP (134 ± 4 mmHg); 4 Sessions per week; 10 min walking outdoors at 4 km.h^{-1} (RPE 11-14)	9	Non-BFR equivalent exercise group	↑ 6MWT ↑ 30STS ↑ TUG ↑ QCST
Cook et al. (2017)	Older adults; 76 ± 10 years	12	12 weeks	Resistance training	6 cm cuff width, 150% SBP (184 ± 25 mmHg); 2 Sessions per week; 3 sets to volitional failure of leg extension, leg curl and leg press at 30% 1RM (50% 1RM for leg press)	1. 12 2. 12	1. HLRT: As per BFR protocol, but at 70% 1RM 2. Control group of light mobility exercises with light resistance	↔ Gait speed ↔ SPPB (no sig. Δ for any measure)
Bryk et al. (2016)	Women with knee osteoarthritis; 61 ± 7 years	17	6 weeks	Resistance training	200 mmHg; 3 Sessions per week; 3 sets of 30 repetitions of seated knee extension at 30% 1RM, in addition to non-occluded resistance exercises for hamstring and gluteal muscles at 70% 1RM	17	Non-BFR equivalent exercise group, with knee extensions performed at 70% 1RM	↔ TUG
Araujo et al. (2015)	Post-menopausal women; 54 ± 4 years	10	8 weeks	Hydrotherapy	18 cm cuff width, 80% LOP (106 ± 10 mmHg); 3 Sessions per week; 4 sets as 30/15/15/15 repetitions of hip flexion/extension, hip abduction/adduction, knee flexion/extension (with 1-5 kg ankle weights)	1. 10 2. 8	1. Non-BFR equivalent exercise group; 2. Inactive control group	↔ STS5 ↔ Gait Speed (no sig. Δ) ↔ Heel-toe walking (no sig. Δ) ↑ TUG

(Continued)

TABLE 1 | Continued

References	Sample (Population, age)	Intervention details				Comparison group(s)		Physical function outcome (BFR vs. comparison)
		n	Duration	Modality	BFR prescription	*n*	Prescription	
Yasuda et al. (2014)	Older adults; 70 ± 7 years	9	12 weeks	Resistance training	5 cm cuff width, 120-270 mmHg; 2 Sessions per week; 4 sets as 30/25/15/10 repetitions of knee extension and leg press, at 20-30% 1RM	10	Inactive control	↑ 30STS
Ozaki et al. (2011)	Older adults; 66 ± 1 years	10	10 weeks	Aerobic training	140-200 mmHg; 4 Sessions per week; 20 min treadmill walking at 4.5 km.h^{-1} and 1.6 degree incline (45% HRR)	8	Non-BFR equivalent exercise group	↔ 30STS ↑ TUG
Abe et al. (2010)	Older adults; 60 - 78 years	11	6 weeks	Aerobic training	160-200 mmHg; 5 Sessions per week; 20 min treadmill walking at 4 km.h^{-1} (45% HRR)	8	Inactive control	↑ 30STS ↑ TUG
Yokokawa et al. (2008)	Older adults; ≥ 65 years	19	8 weeks	Resistance training	4.5 cm width elastic belt, 120% SBP (70-150 mmHg); 2 Sessions per week; Body weight half squats, forward lunges, calf raises, knee lifts, crunches, seated knee flexion and extension	25	Dynamic balance exercise group performing symmetrical and asymmetrical movements; forward and lateral reach; forward and backward steps; standing and walking on a reduced base of support; increasing the complexity of ambulatory tasks; and functional ankle strengthening, all performed on balance mats.	↔ 10mWT ↔ Jump reaction time ↔ Maximum step distance ↑ TUG

Age data presented as mean ± SD unless otherwise indicated. BFR, blood flow restriction pressure; SBP, systolic blood pressure; LOP, limb occlusion pressure; HLRT, High intensity resistance training; 1RM, One-repetition maximum (maximum load able to be lifted for a single repetition); 6RM, Six-repetition maximum (maximum load able to be lifted for six repetitions); HRR, Heart rate reserve; RPE, Rating of perceived exertion; 2MWT, Two-minute walk test; 30STS, 30-second sit-to-stand; TUG, Timed up and go; STS5, Five-times sit-to-stand; 4SST, Four square step test; 6MWT, Six-minute walk test; QCST, Queen's college step test; SPPB, Short physical performance battery; 10mWT, Ten meter walk test.

2016; Clarkson et al., 2017b; Cook et al., 2017; Tennent et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018). However, one of these only reported the minimum compliance rate for inclusion in the analysis (≥66%) and noted a single participant achieved only 37% compliance (although this was removed in the per protocol analysis) (Jorgensen et al., 2018). In a second study, the BFR exercise group had 5 dropouts (∼21%) following initiation of the intervention, compared with 2 from the comparison group (∼7%) (Yokokawa et al., 2008). While these dropouts were not included in the baseline analysis, the overall effect sizes may have been affected by the main intervention group having 25% less participants than the comparison group. Eight of the included studies reported either 100% compliance or a specific percentage of the total exercise sessions completed by participants (Yokokawa et al., 2008; Bryk et al., 2016; Clarkson et al., 2017b; Cook et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018). Compliance ranged from 66% (Jorgensen et al., 2018) to 100% (Bryk et al., 2016; Clarkson et al., 2017b; Ladlow et al., 2018) with a mean compliance rate of 90%. Only three of the included studies identified the intention-to-treat principle when conducting their analyses (Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018).

Reporting Bias

There was no clear indication of reporting bias that may limit the interpretation or applicability of the findings from among the included studies. Minor commentary on the reporting of the main outcome data has been included in the limitations section below.

Other Sources of Bias

Sample size calculations were only presented in seven of the included studies (Araujo et al., 2015; Bryk et al., 2016; Cook et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018). Notably, all studies without sample size calculations had a total number of participants that was below the mean number of participants for included studies within this review. This potentially indicates that many of these studies may have been underpowered. One study which did include sample size calculations indicated that they were going to be underpowered before the study even began (63% power at $\alpha = 0.05$) due to limited access to prospective participants (Jorgensen et al., 2018). Another study used a convenience sample due to time constraints, which is an additional source of bias despite equal randomization within the sample (Ladlow et al., 2018). Other sources of bias included noteworthy acknowledgment of small sample size (Yokokawa et al., 2008; Tennent et al.,

2017), and relatively high functioning participants that may have displayed higher physical function than would be reflective of the broader population in question (Yokokawa et al., 2008; Cook et al., 2017).

Modality and Duration of Interventions

Predominately, exercise training interventions utilized BFR-RT (Yokokawa et al., 2008; Yasuda et al., 2014; Bryk et al., 2016; Cook et al., 2017; Tennent et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018), although three studies employed BFR-AT (Abe et al., 2010; Ozaki et al., 2011; Clarkson et al., 2017b), and one study utilized BFR during hydrotherapy exercises (similar to BFR-RT) (Araujo et al., 2015). Most interventions ranged from 6 to 12 weeks with only one running for a shorter duration of 3 weeks (Ladlow et al., 2018) and no study durations being longer than 12 weeks. Training sessions occurred twice per week in seven studies (Yokokawa et al., 2008; Yasuda et al., 2014; Cook et al., 2017; Tennent et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018), three times per week in two studies (Araujo et al., 2015; Bryk et al., 2016), four times per week in two studies (Ozaki et al., 2011; Clarkson et al., 2017b), with participants in one study completing five sessions per week (Abe et al., 2010), and those in another completing 9 sessions per week and resting on weekends (Ladlow et al., 2018). Of the nine studies that provided an indication of session length, sessions ranged from 8 min duration (Ladlow et al., 2018) to 60 min duration (Jorgensen et al., 2018). Loads for the BFR-RT interventions were generally prescribed at 20–30% 1RM for the exercises completed under occlusion, and generally consisted of approximately 75 repetitions (often as 1 set of 30 repetitions followed by 3 sets of 15 repetitions, which is a common prescription among BFR-RT interventions) (Yasuda et al., 2014; Araujo et al., 2015; Bryk et al., 2016; Tennent et al., 2017; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018). The BFR interventions in the remaining BFR-RT studies utilized training variables closer to traditional HLRT; 3–4 sets of 10–15 repetitions using loads between 40 and 50% 1RM (Yokokawa et al., 2008; Barbosa et al., 2018; Ferraz et al., 2018). Among the three BFR-AT studies included in this review training sessions were 10–20 min in duration and performed at walking speeds of 4–4.5 $km.h^{-1}$ (Abe et al., 2010; Ozaki et al., 2011; Clarkson et al., 2017b), which was noted by the two studies using treadmills as approximately 45% maximal heart rate reserve (Abe et al., 2010; Ozaki et al., 2011), and equivalent to 11–14 on Borg's rating of perceived exertion scale in the outdoor walking study (Clarkson et al., 2017b).

The application of BFR was variable across the included studies. Cuffs were generally pneumatically inflated cuffs capable of regulating pressure, although one study utilized standard tensiometers to apply BFR (Barbosa et al., 2018). Measurements of the cuffs used to apply BFR were detailed in eight of the thirteen included studies, and ranged from 4.5 cm wide (Yokokawa et al., 2008) to 18 cm wide (Araujo et al., 2015). The degree of occlusion and the pressure applied was also variable. Five studies used a relative pressure based on a percentage of total limb occlusion (LOP) (Araujo et al., 2015; Clarkson et al., 2017b; Tennent et al., 2017; Ferraz et al., 2018; Ladlow et al., 2018), or a pseudo-relative pressure based on a percentage of systolic blood pressure (SBP) (Yokokawa et al., 2008; Cook et al., 2017; Barbosa et al., 2018). However, these relative pressures also varied from 60% LOP (Araujo et al., 2015; Clarkson et al., 2017b) to 80% LOP (Tennent et al., 2017), or from 50% SBP (Barbosa et al., 2018) to 150% SBP (Cook et al., 2017). It should be noted that narrower cuffs are generally expected to require greater pressures to achieve the same level of occlusion (Younger et al., 2004; Jessee et al., 2016). However, this relationship is not sufficiently well-characterized, and indeed there is no clear relationship between the degree of LOP and cuff width used among the included studies in this review. Five studies applied an arbitrary pressure range based on pressures used in previous, similar research studies (Abe et al., 2010; Ozaki et al., 2011; Yasuda et al., 2014; Bryk et al., 2016; Jorgensen et al., 2018). Collectively, ten of the thirteen included studies reported the mean pressure or range of progressive pressures used during BFR protocols throughout the study, this ranged from 70 mmHg (Yokokawa et al., 2008) to 270 mmHg (Yasuda et al., 2014).

Outcome Measures

Timed Up and Go

Of the thirteen included studies, eight assessed the timed up and go (TUG) (Yokokawa et al., 2008; Abe et al., 2010; Ozaki et al., 2011; Araujo et al., 2015; Bryk et al., 2016; Clarkson et al., 2017b; Ferraz et al., 2018; Jorgensen et al., 2018). Four of these studies utilized BFR-RT in their interventions (Yokokawa et al., 2008; Bryk et al., 2016; Ferraz et al., 2018; Jorgensen et al., 2018), three utilized BFR-AT (Abe et al., 2010; Ozaki et al., 2011; Clarkson et al., 2017b), and one utilized BFR during hydrotherapy (Araujo et al., 2015). A statistically ($P < 0.05$) and clinically significant decrease in time to complete the TUG was observed in five of these studies and was also a significantly greater decrease in time to complete the TUG (10–15%) than their respective comparison groups (3–6%) (Yokokawa et al., 2008; Abe et al., 2010; Ozaki et al., 2011; Araujo et al., 2015; Clarkson et al., 2017b). Notably, all three of the BFR-AT studies in this review were among these. Two other studies assessing the TUG also saw a significant time effect, however BFR-RT groups were not significantly different to their non-BFR equivalent exercise comparison groups (Bryk et al., 2016; Ferraz et al., 2018).

Sit-to-Stand Tests

The most commonly assessed variation of a sit-to-stand test, the 30 s sit-to-stand test (30STS), was assessed by six of the included studies (Abe et al., 2010; Ozaki et al., 2011; Yasuda et al., 2014; Clarkson et al., 2017b; Ferraz et al., 2018; Jorgensen et al., 2018). Three of these studies utilized BFR-RT as an intervention (Abe et al., 2010; Ozaki et al., 2011; Yasuda et al., 2014; Clarkson et al., 2017b; Ferraz et al., 2018; Jorgensen et al., 2018) and three utilized BFR-AT (Abe et al., 2010; Ozaki et al., 2011; Clarkson et al., 2017b). A statistically significant increase in the number of repetitions completed during the 30STS (14–28%), beyond that of the relevant comparison groups (−2–8%), was observed in three of the six studies (Abe et al., 2010; Yasuda et al., 2014; Clarkson et al., 2017b). One other study demonstrated a main effect for time for both the BFR-AT (increasing repetitions by 4 ± 5)

and non-BFR equivalent exercise comparison group (increasing repetitions by 2 ± 6), but no statistically significant difference between groups (Ozaki et al., 2011).

The five-time sit-to-stand test (STS5) was assessed in a further two of the included studies (Araujo et al., 2015; Tennent et al., 2017). Both studies reported a time effect, whereby time to complete the STS5 decreased regardless of the type of intervention (−27 to −29%) (Araujo et al., 2015; Tennent et al., 2017). However, neither study demonstrated a group by time interaction or a statistically significant difference between groups (Araujo et al., 2015; Tennent et al., 2017).

Gait Speed

Maximal gait speed was assessed in three of the included studies (Yokokawa et al., 2008; Araujo et al., 2015; Cook et al., 2017). Two of these studies utilized BFR-RT as their primary intervention (Yokokawa et al., 2008; Cook et al., 2017), while the other utilized BFR during hydrotherapy (Araujo et al., 2015). None of these studies demonstrated a difference between groups for gait speed. However, one study did demonstrate an overall main effect for time across both intervention groups (Yokokawa et al., 2008). Similarly, one study assessed the change in participant-selected gait speed following a BFR-RT intervention (Tennent et al., 2017). There was a statistically significant time effect (32–37% increase in self-selected gait speed) but no difference between groups (Tennent et al., 2017).

Walking Tests

While there was no single walking test assessed in multiple studies, each of the 6-min walk test (6MWT) (Clarkson et al., 2017b), 2-min walk test (2MWT) (Jorgensen et al., 2018), and the multi-stage locomotion test for endurance (MSLT) (Ladlow et al., 2018) were assessed by individual studies. Following a BFR-AT intervention, the distance covered during the 6MWT improved by a significantly greater amount than the improvement seen following non-BFR walking (9 ± 4% vs. 2 ± 1%; mean ± SD) (Clarkson et al., 2017b). In contrast, there were no differences between BFR-RT and inactive controls for distance covered during the 2MWT, and neither group improved over time (Jorgensen et al., 2018). Finally, healthy adults appeared to significantly improve their performance on the MSLT, and thus their endurance, following a BFR-RT intervention by 29% (increasing by 306 ± 246 m, $P = 0.01$), while there was no improvement from baseline following a HLRT intervention (91 ± 341 m, $P > 0.05$) (Ladlow et al., 2018). However, despite the magnitude of the change in MSLT, the authors of this study indicated that there was no statistical difference between the percentage change observed following BFR-RT compared with HLRT (Ladlow et al., 2018).

Other Measures of Physical Function

A number of additional objective measures of physical function were assessed in only single studies. These included the Four-square step test (4SST) (Tennent et al., 2017), heel-toe walking for balance (Araujo et al., 2015), jump reaction time (following visual stimuli) (Yokokawa et al., 2008), maximum single step distance (Yokokawa et al., 2008), the Queen's College step test (QCST) (Clarkson et al., 2017b), short physical performance battery (SPPB) (Cook et al., 2017), a timed stair ascent (Tennent et al., 2017), handgrip strength (Barbosa et al., 2018) and the Y-balance test (Ladlow et al., 2018). None of the measures of handgrip strength, heel-toe walking for balance or the SPPB showed any significant improvement or difference between groups following BFR-RT or BFR during hydrotherapy (Araujo et al., 2015; Cook et al., 2017; Barbosa et al., 2018). Each of the 4SST, jump reaction time, and maximum step distance were assessed following BFR-RT interventions, and while each of these studies found a main effect for time across all intervention groups, there was no group by time interactions or any statistically significant difference between groups (Yokokawa et al., 2008; Tennent et al., 2017). Of the remaining measures of physical function, the QCST was assessed before and after a BFR-AT intervention (Clarkson et al., 2017b), while both the timed stair ascent and Y-balance test were assessed before and after BFR-RT interventions (Tennent et al., 2017; Ladlow et al., 2018). Both the QCST and timed stair ascent were associated with a main effect for time across both the BFR and non-BFR equivalent exercise interventions in their respective studies (Clarkson et al., 2017b; Tennent et al., 2017), while the study assessing the Y-balance test found no statistically significant change from baseline for their HLRT comparison group (Ladlow et al., 2018). However, all three groups also found a significant group by time interaction, whereby the BFR interventions significantly improved performance on the QCST, timed stair ascent and Y-balance test beyond their respective comparison groups (Clarkson et al., 2017b; Tennent et al., 2017; Ladlow et al., 2018).

DISCUSSION

This systematic review provides evidence supporting BFR exercise as a possible alternative for increasing physical function indicative of ADL. This may be especially important for clinical groups and chronic disease populations for which physical function is a key evaluation of the success of medical and surgical interventions, or valuable in monitoring age- and disease-related physical decline (Groll et al., 2005). The benefit would be especially relevant if the populations in question are contraindicated to the mechanical, and perceptual stress associated with HLRT (Vanwye et al., 2017). Indeed, most of the included studies in this review examine populations that may be contraindicated to HLRT. The majority of included studies examined older adults (and one examining post-menopausal women) at greater risk of falls and with a higher incidence of frailty (Frost et al., 2017), and only one examined otherwise healthy adults. The remaining included studies examined chronic disease populations including end-stage kidney disease and sporadic inclusion body myositis or those in need of musculoskeletal rehabilitation or reconditioning following arthroscopic knee surgery, or with knee osteoarthritis. All of these populations encapsulate individuals with functional deficits for whom "traditional" exercise prescriptions may be too challenging or outright contraindicated, and for whom physical function is a valuable surrogate outcome for the success of

interventions compared with physical performance outcomes such as absolute strength or maximal cardiovascular fitness.

An important factor to consider when interpreting the findings of this review is the sample sizes employed. Among these, one study identified it was only 63% powered due to recruitment limitations (Jorgensen et al., 2018) and while several studies did not provide sample size calculations, two noted that their small sample size may have been a limitation (Yokokawa et al., 2008; Tennent et al., 2017). Suitable power calculations for the outcome measures employed are necessary to ensure the rigor of future research examining measures of physical function. In order to suitably detect small effect sizes the sample size required is markedly larger than that required to detect large effect sizes. For example, if a study comparing the means of two groups is to be 80% powered with an alpha of 0.05, in order to detect a group by time interaction with an effect size of 0.8 requires 52 participants in total (26 per group), but to detect a smaller effect size of 0.3, 352 total participants are required (176 per group). As such, future research must be more conservative in their sample size estimates and target greater recruitment in order to add weight to the discussion of small effect sizes. However, the findings of those studies with small sample sizes in the present review should not be altogether discounted, but instead be more broadly interpreted as a depiction of the potential application of BFR exercise. The majority (nine) of the included studies examined BFR-RT, which is the most widely employed use of BFR (Slysz et al., 2016; Lixandrao et al., 2018), and one examined BFR during hydrotherapy, which is similar to resistance training performed in the water. Across all studies examining BFR-RT the majority reported either a main effect for time across all groups or a group by time interaction, indicating that BFR-RT may be a suitable alternative to other traditional interventions or non-BFR equivalent exercise training for improving physical function. Interestingly, of the studies that were comparing BFR-RT to inactive controls, two found no significant difference between BFR-RT and the control in physical function, but were among those that noted limitations such as being underpowered or having participants that were higher functioning than the majority of the population they were examining, adding weight to the positive findings among other studies supporting BFR exercise as a possible alternative (Cook et al., 2017; Jorgensen et al., 2018). These accounted for more than half the instances in the present review where the BFR intervention was not effective in improving physical function. Perhaps more importantly, due to the physical limitations among populations for which objective physical function is such an important outcome, almost all measures of physical function following BFR-AT improved to a greater extent than the comparison group. The only measure that did not display this group by time interaction in favor of BFR-AT, still showed a time effect, whereby BFR-AT was equally as effective as the non-BFR equivalent intervention for improving 30STS performance (Ozaki et al., 2011). Given the reduced mechanical, haemodynamic and perceptual stress compared with both HLRT or BFR-RT, this suggests that BFR-AT represents a lot of value as an intervention for populations with pronounced physical impairments (May et al., 2017; Neto et al., 2017a; Vanwye et al., 2017).

The two most prominently used measures of physical function among the included studies in this review were the 30STS (Abe et al., 2010; Ozaki et al., 2011; Yasuda et al., 2014; Clarkson et al., 2017b; Ferraz et al., 2018; Jorgensen et al., 2018) and the TUG (Yokokawa et al., 2008; Abe et al., 2010; Ozaki et al., 2011; Araujo et al., 2015; Bryk et al., 2016; Clarkson et al., 2017b; Ferraz et al., 2018; Jorgensen et al., 2018). As such, the collective results from these measures provides the most information regarding the efficacy of BFR exercise for improving physical function in the present review. Performance on the 30STS, considered an indication of functional lower body strength (Jones et al., 1999), improved following BFR exercise interventions in four of the six included studies (by between 14 and 28%) (Abe et al., 2010; Ozaki et al., 2011; Yasuda et al., 2014; Clarkson et al., 2017b), and improved by significantly more than the comparison group in three of these studies (Abe et al., 2010; Yasuda et al., 2014; Clarkson et al., 2017b). This may be expected due to the known ability of BFR exercise to enhance muscle strength and the relative contribution of strength in this measure (McCarthy et al., 2004; Slysz et al., 2016). Similarly, performance on the TUG, a measure of dynamic balance and mobility (Bohannon, 2006), improved following BFR exercise interventions in seven of the eight included studies (by between 10 and 16%) (Yokokawa et al., 2008; Abe et al., 2010; Ozaki et al., 2011; Araujo et al., 2015; Bryk et al., 2016; Clarkson et al., 2017b; Ferraz et al., 2018; Jorgensen et al., 2018), and improved by significantly more than the comparison group in five of these studies (Yokokawa et al., 2008; Abe et al., 2010; Ozaki et al., 2011; Araujo et al., 2015; Clarkson et al., 2017b). Performance on the TUG is known to be inhibited by reduced pelvic, lower limb and core muscle strength (Binda et al., 2003). Therefore, though BFR training primarily affects tissues distal to the restrictive cuff, the common improvement in TUG between studies may suggest that the interventions employed may also enhance the strength of synergist and stabilizer muscles such as pelvic and core musculature (Slysz et al., 2016; Lixandrao et al., 2018). As these measures provide the most insight into the efficacy of BFR exercise for improving objective measures of physical function indicative of ADL among the available literature, there is support for both BFR-RT and BFR-AT for improving physical function. This is especially true given that the reduced intensity and physiological stress of the exercise is suitable for populations that are most in need of improvements in physiological function.

Limitations of the Included Studies

While this review included good quality randomized controlled trials, a moderate risk of bias in some studies was still present (**Figure 2**). Despite an indication of randomization, three studies inadequately reported the methodology with which participants were randomized (Yokokawa et al., 2008; Abe et al., 2010; Yasuda et al., 2014), and only five studies specified the method of allocation concealment (Bryk et al., 2016; Clarkson et al., 2017b; Tennent et al., 2017; Barbosa et al., 2018; Ladlow et al., 2018). Perhaps most notably, blinding of participants and study personnel was of concern (Schulz et al., 1995). However, blinding of participants is something that may be difficult to account for with training studies, particularly when the comparison

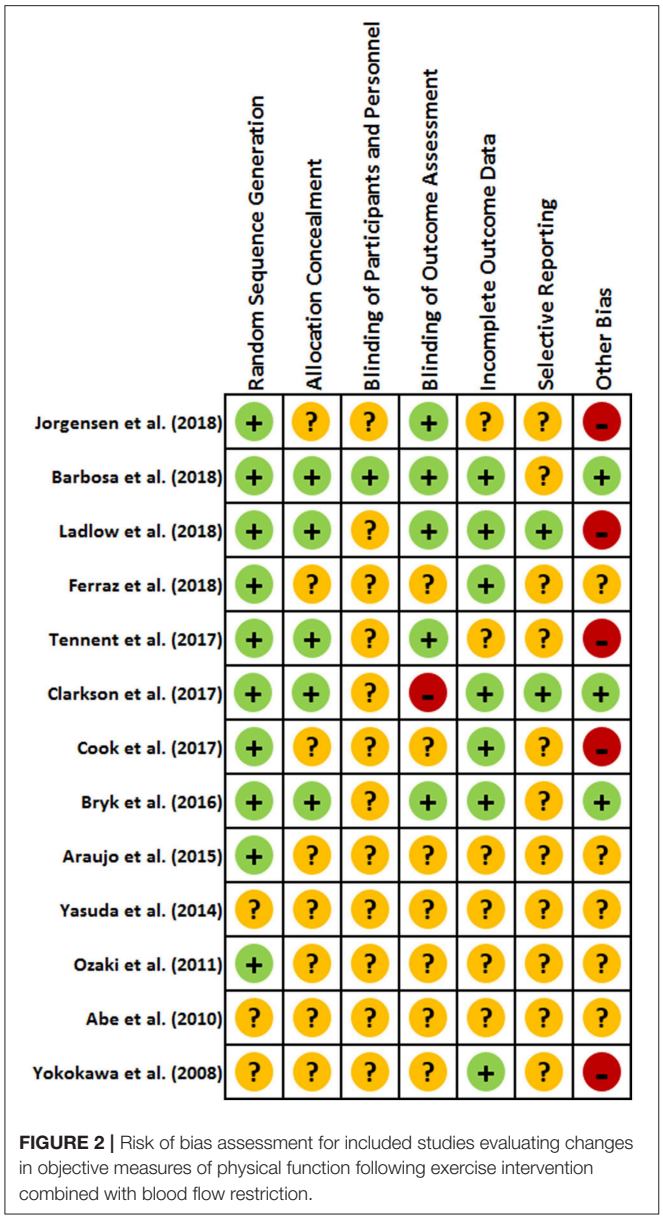

FIGURE 2 | Risk of bias assessment for included studies evaluating changes in objective measures of physical function following exercise intervention combined with blood flow restriction.

group is either a different format of exercise, or an inactive control and is inherent in many training studies. Additionally, only three studies indicated the use of the intention-to-treat principle (Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018); only seven studies included sample size calculations (Araujo et al., 2015; Bryk et al., 2016; Cook et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018); and only eight sufficiently reported compliance (Yokokawa et al., 2008; Bryk et al., 2016; Clarkson et al., 2017b; Cook et al., 2017; Barbosa et al., 2018; Ferraz et al., 2018; Jorgensen et al., 2018; Ladlow et al., 2018). Two studies presented only the mean and variance of the change in measures of physical function from before to after the intervention, but did not report the means and variance for both the pre- and post-intervention time points (Cook et al., 2017; Ladlow et al., 2018). This potentially limits the understanding of how impactful the demonstrated change scores were, and may limit the interpretation of how low the level of physical function must be in order to garner an advantage from this training modality.

From a broader perspective, the overall low number of studies and lack of homogeneity among them makes it difficult to collectively analyse and interpret the results for many of the less commonly used measures of physical function. However, the commonly used measures in the 30STS and TUG provide substantial evidence supporting the efficacy of BFR exercise for improving physical function, particularly functional strength, dynamic balance and mobility. This may indicate that future studies need to assess the influence of BFR training interventions on performance of a more holistic battery of measures of physical function. The lack of homogeneity among included studies is also the primary reason a meta-analysis was not attempted in addition to the present review. Among the thirteen included studies, there were seven different populations examined. While exercise may generally have a similar effect, there are likely physiological differences between these different populations that are extraneous variables that make it difficult to provide generalizations about BFR exercise for any single population. This is the primary reason for why reporting minimal clinically important difference (MCID) is difficult for the outcomes in this review, as this is something that can be markedly variable depending on the population in question, and to try to imply a blanket MCID across multiple populations may be misleading. This would be a valuable addition to the reporting of the outcomes for objectively measured physical function, and future research should provide an indication of what is considered a MCID for the populations they assess. Likewise, large variability in the exercise prescriptions (exercise training modalities, pressure applications and restriction durations) used among the included studies makes it difficult to elucidate whether any single prescription is particularly useful or more efficacious than another. However, it is difficult to recommend specific exercise prescriptions as this is variable in response to the population group being examined, and the training objectives of note. Perhaps a more apt recommendation is for more repetition of similar exercise prescriptions for specific training objectives among single populations as a means of enhancing evidence that is touched on by previous findings. Finally, while the 30STS and TUG were more frequently examined, making it easier to draw conclusions about the general effects of BFR exercise and performance on these measures, no other single measure of physical function identified in this review was assessed in more than two studies. Thus, more studies examining similar key measures of physical function are required when assessing outcomes from BFR training.

CONCLUSIONS

Physical function is generally an undervalued and infrequently measured outcome among blood flow restriction exercise

studies, which traditionally focus on muscle mass and strength outcomes. Task-specific measures of physical function indicative of activities of daily living may be more clinically relevant and applicable for evaluating the management or progression among chronic disease and other clinical populations (Groll et al., 2005). The results of this review indicate that blood flow restriction exercise has potential for improving physical function measured by tasks reflective of everyday activities. However, the inconsistency regarding target populations, exercise prescription, and outcome measures assessed demonstrates a need for greater research focus and consistency, particularly within specific populations in future research. Regardless, blood flow restriction exercise is frequently purported to be of significant benefit for chronic disease and other clinical populations due to reduced mechanical, haemodynamic and perceptual stress. In addition to these reduced physiological stresses, the present review suggests that blood flow restriction exercise may provide equivalent, or even greater stimulus for improving physical function than some non-blood flow restriction equivalent or more traditional exercise prescriptions. Therefore, this review supports the utilization of blood flow restriction exercise in clinical rehabilitation or the management of chronic diseases, particularly with regard to improving measures of physical function indicative of everyday tasks that utilize strength, dynamic balance and mobility.

AUTHOR CONTRIBUTIONS

MC was primarily responsible for the conception of and rationale for the review. MC, AM, and SW contributed to the search strategy, the selection criteria, the review, and writing of the manuscript. MC and AM completed the database searches, study selection, and assessment of bias. MC completed data extraction and compilation of results.

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Muscular Adaptations to Whole Body Blood Flow Restriction Training and Detraining

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Resistance training with blood flow restriction is typically performed during single exercises for the lower- or upper-body, which may not replicate *real world* programming. The present study examined the change in muscle strength and mass in a young healthy population during an 8-week whole body resistance training program, as well as monitoring these adaptations following a 4-week detraining period. Thirty-nine participants (27 males, 12 females) were allocated into four groups: blood flow restriction training (BFR-T); moderate-heavy load training (HL-T), light-load training (LL-T) or a non-exercise control (CON). Testing measurements were taken at Baseline, during mid-point of training (week 4), end of training (week 8) and following four weeks of detraining (week 12) and included anthropometrics, body composition, muscle thickness (MTH) at seven sites, and maximal dynamic strength (1RM) for six resistance exercises. Whole body resistance training with BFR significantly improved lower- and upper-body strength (overall; 11% increase in total tonnage), however, this was similar to LL-T (12%), but both groups were lower in comparison with HL-T (21%) and all groups greater than CON. Some markers of body composition (e.g., lean mass) and MTH significantly increased over the course of the 8-week training period, but these were similar across all groups. Following detraining, whole body strength remained significantly elevated for both BFR-T (6%) and HL-T (14%), but only the HL-T group remained higher than all other groups. Overall, whole body resistance training with blood flow restriction was shown to be an effective training mode to increase muscular strength and mass. However, traditional moderate-heavy load resistance training resulted in greater adaptations in muscle strength and mass as well as higher levels of strength maintenance following detraining.

Keywords: strength training, hypertrophy, vascular occlusion, rehabilitation, BFR, resistance exercise

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INTRODUCTION

Training using blood flow restriction exercise (BFRE) is gaining popularity among researchers and practitioners such as medical staff, physiotherapists, strength and conditioning coaches and rehabilitation specialists (Patterson and Brandner, 2017). In general, the increase in muscle strength and mass with BFRE is greater than lifting light loads (≤40% 1 repetition maximum [1RM]) without BFR, while often being closely matched to moderate-heavy load (≥65% 1RM) resistance training (Lixandrão et al., 2017). BFR resistance training may provide several advantages over traditional

moderate-heavy load resistance training, for example, these muscular adaptations are achieved despite lower relative external loads, produce less muscle damage and thus training frequency can be increased, while muscle hypertrophy has also been shown in as little as 1–2 weeks (Scott et al., 2014). For these reasons, healthy/general populations may be recommended to perform BFRE as part of a training program in order to increase muscular strength, mass, and functional performance or activities of daily living.

BFR has been combined with several different single-joint lower-body (e.g., knee extension, knee flexion, ankle plantarflexion) and upper-body exercises (e.g., elbow flexion, elbow extension) (Patterson and Brandner, 2017), as well as compound multi-joint exercises such as the squat and bench press (Abe et al., 2012). However, the examination of a training program using a limited number of exercises (e.g., one or two) does not reflect typical applied resistance training programs that instead comprise one or more exercises for multiple anatomical regions (American College of Sports Medicine, 2009). Previous studies that have examined multiple resistance exercises with BFR are diverse and include lower-body exercises only (Sumide et al., 2009), upper-body exercises only (Thiebaud et al., 2013), bodyweight exercises (Yokokawa et al., 2008), in-water exercises (Araújo et al., 2015), older adult populations only (Karabulut et al., 2010; Bryk et al., 2016), or clinical cases (Jørgensen et al., 2015; Tennent et al., 2016). With only two of these comparing a BFR training group against traditional moderate-heavy load training (HL-T) in older adults (Karabulut et al., 2010; Bryk et al., 2016). Therefore, a "real world" full-body BFR training program incorporating both upper- and lower-body exercises using traditional weights or weight machines while comparing against a non-BFR control group and a heavy-load resistance exercise group is yet to be investigated.

One other aspect that has been infrequently examined is the effect of detraining on muscular adaptations following BFR training. Detraining is commonly associated with strength loss, muscular atrophy, and reductions in functional capacities in both older adults and clinical populations (Mujika and Padilla, 2000). Physical training in general populations may cease due to injury or illness, time/effort, boredom or fatigue. For athletes, there may be interruptions in training due to competition, travel, offseason rest or taper strategies, or other factors (Mujika and Padilla, 2000). Therefore, while there is an abundance of training studies examining muscular adaptations to BFRE, only few have examined the effects of detraining. Results from these studies have produced mixed results that likely stem from examining different combinations of young adults (Yasuda et al., 2014b,c), older adults (Yasuda et al., 2014a), and the effects of detraining following only lower-body (Yasuda et al., 2014a) or only upper-body training (Yasuda et al., 2014b,c, 2015). From the available literature, muscle strength and some aspects of muscle cross sectional area (CSA) have been found to be maintained for up to 12–24 weeks in older adults, but not differently to non-BFR training or control (non-exercise) group (Yasuda et al., 2014a, 2015). The only study to compare against moderate-HL-T found that after a 3 week detraining period, both the BFR and moderate-heavy load group maintained 1RM bench press strength, but only the moderate-heavy load group maintained pectoralis major and triceps brachii CSA (Yasuda et al., 2014b).

Therefore, the aim of the present study was to investigate the time course adaptations to training (8 weeks) and detraining (4 weeks) in muscle strength and mass to a full-body BFR resistance training program and compare these results to a traditional moderate-heavy load resistance training program and a light-load resistance training (without BFR).

MATERIALS AND METHODS

Participants

Thirty-nine healthy participants (27 males and 12 females; see **Table 1** for participant characteristics) volunteered to take part in the study and provided written and informed consent to the experimental procedures. Participants had no known history of peripheral or neurological impairment, cardiovascular, pulmonary, or metabolic disease, musculoskeletal injuries, or self-reported smoking. Additionally, none of the participants had involvement in any kind of resistance training in the previous 2 months. Additionally, participants were asked to refrain from additional exercise and only complete incidental physical activity outside of the study.

Sample Size

Study sample size was determined by undertaking a power analysis (G*Power v 3.1.9.2). Sample size for the present study was primarily based on lower limb muscle strength, due to a lack of comparative whole body BFR training studies. This was derived from the mean changes observed in previous studies investigating muscular adaptations following knee extension or knee flexion exercise with BFR (Fujita et al., 2008; Karabulut et al., 2010; Kacin and Strazar, 2011). The expected mean changes in strength were approximately 11% for BFR-T, 16% for HL-T, 2% for LL-T, and 0% for CON with a pooled standard deviation of 13%. Target statistical power was set to $\geq$0.80 to detect significant increases in muscle strength at $P \leq 0.05$. This analysis suggested that 32 participants would be required across 4 groups, or 8 per group. However, given that this is estimated of single joint training studies, and not whole body exercise, a more conservative 10 per group was targeted.

TABLE 1 | Anthropometric characteristics as measured at baseline.

	BFR-T (*n* = 11)	HL-T (*n* = 11)	LL-T (*n* = 10)	CON (*n* = 7)
Gender (M, F)	8, 3	7, 4	7, 3	5, 2
Age (years)	23 ± 3	23 ± 3	23 ± 3	24 ± 2
Height (cm)	175.7 ± 12.2	171.5 ± 8.9	177.0 ± 14.0	183.2 ± 8.0
Body mass (kg)	72.5 ± 15.5	71.1 ± 12.0	74.5 ± 21.1	77.5 ± 12.6
BMI (kg.m^{-2})	24 ± 3	24 ± 3	23 ± 4	23 ± 3

BMI, body mass index; BFR-T, blood flow restriction training; CON, control; HL-T, heavy-load resistance training; LL-T, light-load resistance training.

Experimental Design

Participants were familiarized with all testing and exercising protocols 3–7 days prior to beginning the study. Testing was conducted prior to training (baseline), at mid-point of training (week 4), end of training (week 8), and following a four-week detraining period (week 12). Therefore, the total duration of the experimental study was 13 weeks. During the training and detraining period, participants were asked to maintain their normal diet and physical activity levels. Following baseline testing, participants were allocated to one of three training groups (total n = 32) or a non-training control group (n = 7). To minimize covariate imbalance, participants in each group were matched for gender and pre-training knee extension (KE) strength to facilitate equal distribution. However, this method does not necessarily eliminate bias or unknown factors (e.g., other covariates). Testing measurements included anthropometrics (height and body mass), body composition using Dual X-ray Absorptiometry (DXA) and ultrasound to measure muscle thickness (MTH) at seven sites, and maximal dynamic strength (1RM) for six resistance exercises. Testing was completed in this order, with visits lasting for approximately 2–2.5 h. The primary investigator was present to supervise all training sessions throughout the experiment and was assisted by two students of the school.

Resistance Training Protocols

Participants in the resistance training groups performed 20 training sessions across 8 weeks, three times per week on non-consecutive days. If a participant was unable to attend a scheduled training session, the session was completed elsewhere within the training week. Thus, compliance to the 20 training sessions across 8 weeks was 100%. All training sessions comprised three lower- and three-upper body exercises. Prior to beginning each training session, participants completed a standardized warm up consisting of 5 min cycling on a Monark cycle ergometer (50–100 W). Subsequently, participants began training and completed the exercises in the following order; knee extension (KE), barbell back squat (SQ), calf raise (CR) on a 45° leg press, barbell bench press (BP), seated row (SR), and barbell biceps curl (BC). The loads and repetitions performed for all training groups were different, however, there were some similarities. For all training groups, four sets were performed for KE as an equivalent to the standard four sets performed for BFR training. Only three sets were performed for each of the remaining exercises. Between the three lower- and three upper-body exercises there was a 5-min recovery period, which was approximately the time it would take for the BFR group to deflate and remove lower-body cuffs and then reapply and inflate upper-body cuffs. Loads for training sessions 1–10 were calculated as a percentage of 1RM measured during Baseline strength testing, and sessions 11–20 from the week 4 strength testing session. All repetitions for all resistance exercises for all training groups was monitored by a metronome with a repetition timing of 2 s for the concentric phase and 2 s for the eccentric phase. The total training duration for each training session was approximately 45 min.

Heavy-Load Training

Participants in the HL-T group (n = 11; 3 females) were required to exercise at 70% 1RM. For KE, participants performed four sets of 8–10 repetitions, separated by 1-min rest between sets. Following this, participants completed the additional five resistance exercises, but were only required to complete three sets of 8–10 repetitions. There was a 1-min recovery period between all exercises and sets.

Light-Load Training

Participants in the LL-T group (n = 10; 3 females) were required to exercise at 20% 1RM. For KE, participants performed a total of 30 repetitions in the first set, followed by three sets of 15 repetitions separated by 30 s rest between sets. Following this, participants completed the additional five resistance exercises, but were only required to complete three sets of 15 repetitions.

This set x repetition scheme has been used in previous BFRE studies of similar duration and using multiple exercises (e.g., Weatherholt et al., 2012) and shown to produce increases in muscle strength and size following training. There was a 30 s rest period between sets, and a 1-min recovery period between exercises.

Blood Flow Restriction Training

Participants in the BFR-T group (n = 11; 3 females) were required to perform the same resistance training program as described for LL-T. However, all resistance exercises were completed with BFR at 60% of each individuals limb occlusion pressure (LOP) as previously described from our laboratory (May et al., 2018). Briefly, BFR was applied using an automatic tourniquet system (ATS 3000, Zimmer Inc., OH, United States) connected to inflatable pneumatic cuffs. Cuff widths and lengths for the lower-body (86 cm long, 10.5 cm wide, bladder length 80 cm, bladder width 8 cm) and upper-body (52 cm long, 10.5 cm wide, bladder length 45 cm, bladder width 8 cm) were used throughout the duration of the study. Prior to beginning each training session, participants in the BFR group were fitted with cuffs to the most proximal portion of each thigh in order to perform all three lower-body resistance exercises. The final exercising BFR pressure was set immediately prior to KE and was maintained continuously throughout all three lower-body resistance exercises (approximately 16 min) before the cuffs were deflated. Participants were then given a 5-min recovery time and fitted with cuffs to the most proximal portion of their upper arms. The final exercising BFR pressure was set immediately prior to performing the BP exercise and was maintained continuously throughout all three upper-body resistance exercises (approximately 14 min) before cuff deflation.

To provide individualized restriction pressures, LOP was determined separately for each lower- and upper-body limb for each participant prior to beginning the resistance training protocol (baseline) and at week 4 prior to beginning the second block of training. All measurements were taken in a seated position, as this position was most closely related with the primary outcome exercise (KE), and it has been recommended to measure LOP in the most exercise specific body position

(Hughes et al., 2018). With the restriction cuffs in place on the limb, a plethysmograph (LOP Sensor Kit, Zimmer Inc., OH, United States) was applied to the distal process of the second phalange of the foot or hand (second toe/finger) for lower- and upper-body, respectively. Automated measurement of LOP was performed using the inbuilt LOP function (ATS 3000, Zimmer Inc., OH, United States), whereby the restriction cuffs gradually inflated to produce a continuous rise in pressure until tissue blood flow was no longer detected at the measurement site. Measurement of LOP was conducted twice on each limb and were typically within 20 mmHg, whereby the average was then used to set the cuff pressure for exercise. If the measurements were more than 20 mmHg apart on each limb, a third test would be conducted and an average of all three tests would be taken. LOP measures taken at baseline and week 4 of the lower-body (180 ± 7 and 181 ± 8 mmHg, respectively) and upper-body (133 ± 3 and 136 ± 5 mmHg, respectively) were not significantly different. Once LOP was determined, the restriction pressure was set at 60% LOP, equal to 107 ± 5 and 109 ± 5 mmHg for the lower-body and 80 ± 2 and 81 ± 3 mmHg for the upper-body for training sessions 1–10 and 11–20, respectively.

Control

Participants in the CON group (n = 7; 2 females) performed all testing sessions across the duration of the study and were requested not to engage in additional physical activity or exercise outside of their normal daily routine whilst maintaining their normal diet during the study period.

Anthropometrics

Height and body mass were measured to the nearest 0.5 cm and 0.1 kg, respectively, using a stadiometer (220 portable stadiometer, Seca, Hamburg, Germany) and digital electronic scale (UC-321, A&D Co. Ltd., United States). Body mass index (BMI) was then calculated as; BMI ($kg.m^{-2}$) = Body mass (kg)/Height^2 (m).

Muscle Strength and Mass

Maximal Strength

Dynamic 1RM testing was performed for the lower-body (knee extension, barbell back squat, calf raise) and upper-body (barbell bench press, seated cable row, barbell biceps curl). Participants completed all 1RM tests through a full range of motion. Before each 1RM test, participants warmed up at 50% of their estimated 1RM. Single repetition lifts were conducted with progressively heavier loads until failure, defined as the final load that could be successful lifted with correct technique where an additional 0.5–5.0 kg could not be successfully lifted. Rest intervals between 1RM attempts were dependent on participant readiness but ranged from 2–5 min, while not more than four to six attempts were completed during any test. During testing, 1RM attempts were alternated between the lower-body and upper-body exercises in order to minimize accumulated fatigue.

Body Composition

Dual energy X-ray absorptiometry (DXA; Lunar Prodigy, GE Lunar Corp., Madison, WI, United States) using software version 12.30.008 was used to assess total bone-free lean body mass (LM), total bone-free fat mass (FM), as well as arm-LM, leg-LM, and trunk-LM using a total body scan. Calibration of the DXA was performed on each testing day prior to scanning participants, and scanning procedures were standardized for all participants and done in accordance with recently published best practice protocols for the assessment of whole body composition (Nana et al., 2015). In addition, all analysis of DXA was undertaken by the primary investigator for consistency, who was not blinded to the training group of the participant. The short-term coefficient of variation measured on two consecutive days for repeated measurements of total body lean mass and fat mass in our laboratory ranges from 1.0 to 1.7%. Participants were placed in a supine position with arms placed close to the sides of the body in a neutral position within the 60 cm scanning area on the DXA table. Velcro straps were placed around the ankles to hold the legs together during the scans and prevent any movement.

Ultrasound Muscle Thickness

B-mode ultrasonographic evaluation of skeletal MTH was taken at seven sites from the anterior and posterior aspects of the body using a Sonosite ultrasound (Springfield, NJ, United States). All measurements were taken on the participants' dominant side with subjects lying in supine and prone positions. A 5–15 Hz scanning transducer head was lubricated with transmission gel and placed lightly on the marked area without depressing the dermal surface. Distortion of tissue due to excess compression was eliminated by observing that no movement of the tissue occurred in the real-time ultrasound image. When a clear image was visible on the monitor, the image was captured for immediate analysis. A total of six measurements were taken for each of the anatomical sites (listed below) and the average was used for analysis. The short-term coefficient of variation for repeated measurements on two trials ranged from 1.3 to 6.4%. MTH was determined as the distance between the adipose-muscle interface and muscle-bone interface from the ultrasound image in accordance as per previous protocols (Abe et al., 1994). Briefly, the seven anatomical landmarks of the sites were as follows; *Biceps and triceps:* on the anterior and posterior surface equal to 60% distal between the lateral epicondyle of the humerus and the acromial process of the scapula; *Pectoralis major:* at the clavicular midpoint and between the third and fourth costa; *Quadriceps and hamstring:* on the anterior and posterior surface midway between the lateral condyle of the femur and the greater trochanter; *Gastrocnemius and tibialis anterior:* on the anterior and posterior surface equal to 30% distal of the lateral condyle of the tibia and the lateral malleolus of the fibula. To ensure accuracy of the data across all testing time points, the marking sites were recorded and matched on each testing session.

Statistical Analysis

All data for measured variables were found to be normally distributed as assessed with a Shapiro-Wilks test ($P \leq 0.05$). All dependent variables for muscle strength, body composition, and MTH were analyzed for absolute (kg) as well as normalized percentage change from baseline which was calculated as: $(post - pre)\,/pre\ x\ 100$. Total tonnage (TT) was calculated as a

sum of all six resistance exercises 1RM at each testing time point, as a reflection of whole body strength. One outlier was removed from the normalized data for CR as the data was 3SD above the mean. A linear mixed model was used to measure main effects for Group (BFR-T, HL-T, LL-T, CON) and Time (Baseline, week 4, week 8, week 12) while also accounting for the small sample size and missing data points. For any Group x Time interactions, a Bonferroni correction was used to determine differences for each dependent variable while accounting for family-wise error. The linear mixed model was performed using SPSS statistical software (v25). The level of significance was set at $P \leq 0.05$ and all data is presented as mean ± standard deviation (SD) unless stated otherwise.

RESULTS

There were no significant differences between groups for age, height, body mass, and BMI (**Table 1**). There was one injury recorded in the HL-T group, with one participant reporting a lower back complaint following training sessions when performing the squat exercise. Therefore, the SQ was removed from their training and all subsequent test sessions whereby their results were not included in the analysis for the SQ or TT. There were no other injuries recorded in any of the other training groups, and no side effects reported for anyone performing BFR exercise (both acute or chronically). One participant in HL-T and one in LL-T were unable to attend the detraining test week and thus this data was not included in the analysis at this time point.

Training Adaptations

Lower-Body Maximal Strength

Overall, for absolute strength (kg) for KE, SQ, and CR there were no significant main effects for Group (P = 0.50–0.94; **Table 2**). However, there were significant main effects for Time ($P < 0.0001$; **Table 2**), and significant interactions (Group x Time) for both SQ and KE ($P < 0.05$–0.0001). As such, CON

TABLE 2 | Absolute (kg) change in 1RM strength.

	BFR-T	HL-T	LL-T	CON
Lower-body strength				
Knee extension				
Baseline	75.58 ± 23.14	79.26 ± 19.61	78.79 ± 20.13	87.47 ± 20.47
Week 4	86.57 ± 30.25*	90.58 ± 21.99*	85.83 ± 24.92	87.87 ± 22.47
Week 8	91.40 ± 32.50*	99.05 ± 24.77*	89.72 ± 26.60*	84.30 ± 19.19
Week 12	89.26 ± 32.00*	96.25 ± 28.91*	87.40 ± 27.59	85.16 ± 20.45
Back Squat				
Baseline	82.05 ± 22.20	80.68 ± 19.88	65.00 ± 17.83	85.00 ± 33.17
Week 4	87.27 ± 21.17	86.00 ± 20.55	73.00 ± 17.23*	85.71 ± 33.81
Week 8	90.68 ± 22.19*	93.75 ± 20.92*	79.75 ± 20.50*	88.93 ± 32.17
Week 12	89.55 ± 23.71*	84.16 ± 20.04*	80.83 ± 21.65*	85.36 ± 33.12
Calf raise				
Baseline	200.00 ± 50.22	175.00 ± 58.01	167.78 ± 45.49	188.57 ± 52.66
Week 4	211.50 ± 55.18	195.45 ± 55.16*	183.61 ± 51.22*	188.57 ± 52.97
Week 8	217.25 ± 57.96*	206.14 ± 54.26*	185.83 ± 49.31*	190.71 ± 49.87
Week 12	206.67 ± 55.45	197.50 ± 53.35*	173.13 ± 48.62	193.57 ± 57.28
Upper-body strength				
Bench press				
Baseline	55.32 ± 17.40	55.00 ± 17.54	49.25 ± 17.52	64.29 ± 22.99
Week 4	57.91 ± 18.04	58.18 ± 19.43	52.35 ± 17.95	65.00 ± 23.63
Week 8	58.46 ± 14.27	62.27 ± 20.23*	53.10 ± 18.13*	65.21 ± 24.15
Week 12	55.64 ± 17.53	57.25 ± 21.49	51.11 ± 19.13	66.43 ± 23.36
Seated row				
Baseline	52.07 ± 17.04	50.30 ± 12.79	49.68 ± 13.04	58.54 ± 17.38
Week 4	53.55 ± 17.00	54.39 ± 13.20*	50.68 ± 13.47	59.46 ± 15.30
Week 8	54.95 ± 16.13*	58.05 ± 13.72*	51.50 ± 13.27*	58.18 ± 16.99
Week 12	53.77 ± 16.51	55.30 ± 13.53*	49.61 ± 13.86	58.93 ± 15.67
Biceps curl				
Baseline	27.82 ± 9.37	27.68 ± 7.03	27.75 ± 10.17	33.36 ± 12.49
Week 4	30.14 ± 9.66*	29.77 ± 6.85*	28.75 ± 9.32	34.29 ± 12.97
Week 8	30.95 ± 9.78*	31.36 ± 7.35*	28.80 ± 8.79	35.50 ± 13.77*
Week 12	31.05 ± 9.47*	30.15 ± 7.95*	29.28 ± 9.08	35.00 ± 13.15

** indicates significantly different from Baseline. BFR-T, blood flow restriction training; CON, control; HL-T, heavy-load resistance training; LL-T, light-load resistance training.*

remained similar to baseline across time for all exercises, while in general absolute strength for HL-T, BFR-T, and LL-T increased across time for all exercises (**Table 2**). Examination of the normalized data (%) elucidated a main effect for both Group ($P < 0.01$) and Time ($P < 0.0001$), and a Group x Time interaction ($P < 0.0001$) for percentage change in KE 1RM strength (**Figure 1A**). At week 4 and 8 the percentage increase in KE strength was greater in both BFR-T and HL-T compared with CON ($P < 0.05$). The percentage change for HL-T was also significantly greater than LL-T ($P < 0.0001$). KE was only increased at week 8 for LL-T. For SQ normalized data (%), main effects for Group ($P < 0.01$) and Time ($P \leq 0.0001$) were identified. A Group x Time ($P < 0.01$; **Figure 1B**) interaction was also detected whereby the percentage increase in SQ 1RM strength was greater for HL-T and LL-T at week 4 ($P \leq 0.05$), and while the percentage increase was also higher for all groups

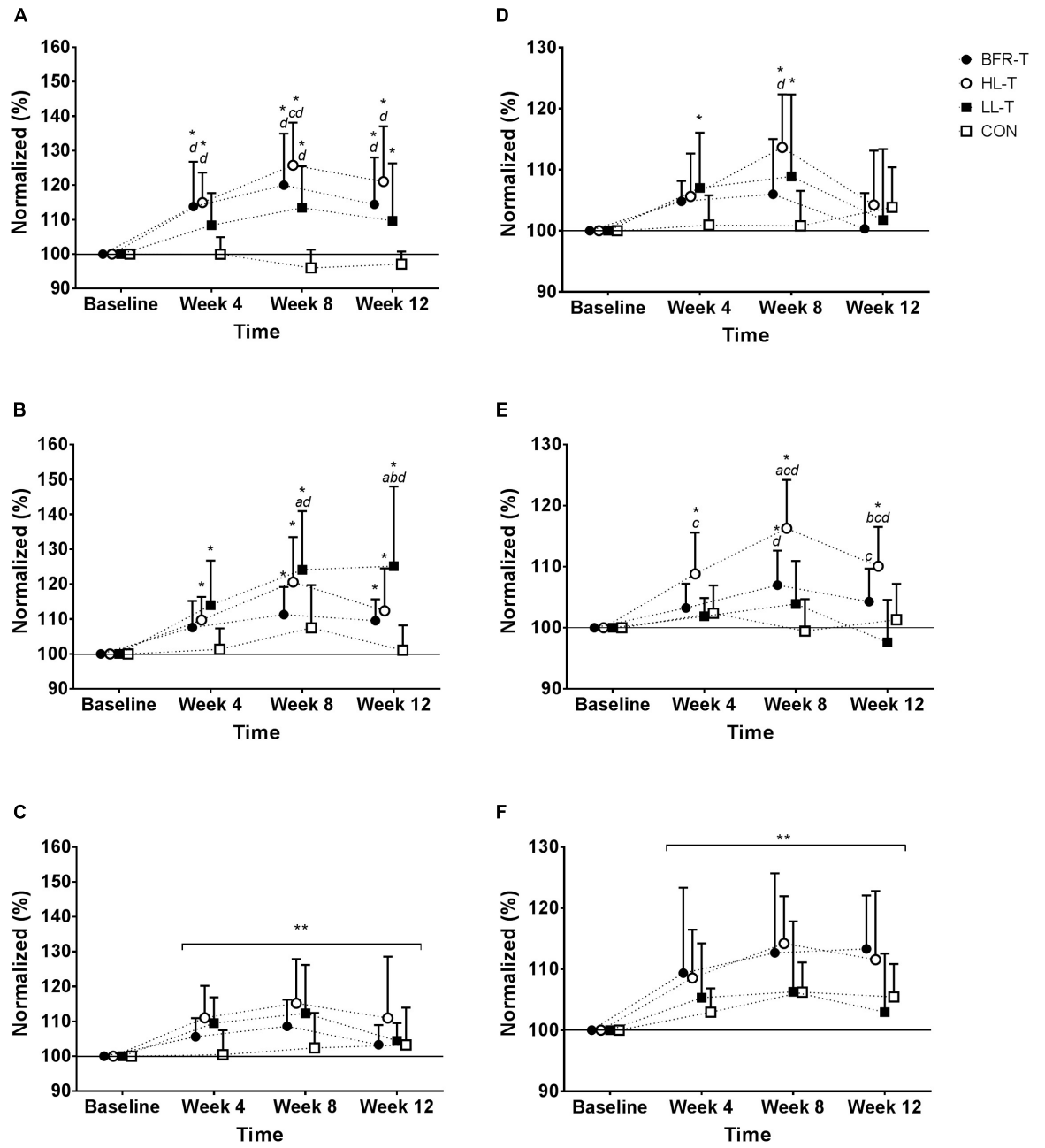

FIGURE 1 | Normalized (%) change in 1RM strength for each exercise. Knee extension **(A)**, Back squat **(B)**, Calf raise **(C)**, Bench press **(D)**, Seated row **(E)**, and Biceps curl **(F)**. * indicates significant difference within Group compared with Baseline ($P \leq 0.05$); ** significant main effect for Time ($P \leq 0.05$); *a* significantly different to BFR-T; *b* significant different to HL-T; *c* significantly different to LL-T; d significantly different to CON.

except CON at week 8, only the percentage increase for LL-T was higher in comparison with BFR-T and CON. There was no main effect for Group, and no Group x Time interaction detected for CR, only a main effect for Time ($P \leq 0.0001$; **Figure 1C**).

Upper-Body Maximal Strength

Overall, absolute strength (kg) for BP, SR, and BC there were no significant main effects for Group (P = 0.53–0.70; **Table 2**). However, there were significant main effects for Time ($P < 0.0001$), as well as significant Group x Time interactions for BP and SR (P = 0.05–0.0001). The CON group remained similar to baseline across time for all exercises. In general, upper-body strength increased across time for BP among all groups except CON and SR for HL-T only (**Table 2**). Examination of the normalized data (%) for percentage change in BP 1RM strength revealed no main effect for Group (P = 0.53), but there was for Time ($P < 0.0001$). Additionally, there was a Group x Time interaction ($P < 0.05$; **Figure 1D**). At week 8, the percentage increase from baseline in BP strength for HL-T and LL-T was significant, but only HL-T was greater than CON. For SR normalized data (%), main effects for Group ($P < 0.0001$) and Time ($P < 0.0001$) were found. A Group x Time ($P < 0.0001$; **Figure 1E**) interaction was also detected whereby the percentage increase in SR 1RM was increased at week 4 for HL-T only, which was also higher than LL-T. At week 8, the percentage change from baseline was significant for BFR-T and HL-T, and while BFR-T was higher than CON, the percent increase for HL-T was significantly greater than all other groups. There was no main effect for Group, and no Group x Time interaction detected for absolute (kg) or normalized percentage change in BC 1RM, however, there was a main effect for Time ($P < 0.0001$; **Figure 1F**).

Total Tonnage

Overall, for absolute TT (kg) there was no main effect for Group (P = 0.75). However, there was a main effect for Time ($P < 0.0001$), and a Group x Time interaction ($P < 0.0001$; **Figure 2A**). There was no change in TT for the CON group throughout training, whereas all other groups increased TT at week 4 and BFR-T and HL-T further increased TT at week 8 (**Figure 2A**). When examining the normalized data (%) for TT, there was a significant main effect for Group and Time, and a Group x Time interaction (all $P \leq 0.0001$; **Figure 2B**). The percentage increase in TT at weeks 4 and 8 was significant for BFR-T, HL-T, and LL-T but not CON. At week 4, the percentage increase was higher for HL-T in comparison with CON. At week 8, the percentage increase was higher for HL-T in comparison with all other groups, and the percentage increase was higher for both BFR-T and LL-T compared with CON.

Body Composition

Table 3 displays the body composition (kg) values as represented at Baseline as well as a summary of the main effects and interactions. For access to the full absolute (kg) and normalized (%) body composition changes see **Supplementary Tables 1**, **2**, respectively. When examining the normalized data (%) for body composition, there we no main effects for Group reported, nor any Group x Time interactions. However, there were significant main effects for Time whereby at week 8 all groups showed similar increases in LM, arm-LM, and leg-LM (**Table 3** and **Supplementary Tables 1**, **2**).

Muscle Thickness

Table 3 displays the MTH (cm) values as represented at Baseline as well as a summary of the main effects and interactions. For the full data for absolute (kg) and normalized (%) MTH changes see **Supplementary Tables 3**, **4**. For absolute (cm) MTH, there were no main effects for Group at any measurement site (all $P > 0.05$). However, a significant main effect for Time was detected for all sites ($P < 0.01$) whereby in general MTH increased across the duration of the training program, except for Tibialis Anterior and Pectoralis Major. A Group x Time interaction was detected for Biceps and Quadriceps MTH only ($P < 0.05$). For Biceps MTH, both BFR-T and HL-T were significantly increased at week 4 relative to Baseline, and only HL-T was significantly increased at week 8, with no other changes reported for the other groups. For Quadriceps MTH, BFR-T, HL-T, and LL-T were significantly increased at week 8 relative to baseline.

A similar pattern was found when examining the normalized data (%) for MTH, with main effects for Time reported for all

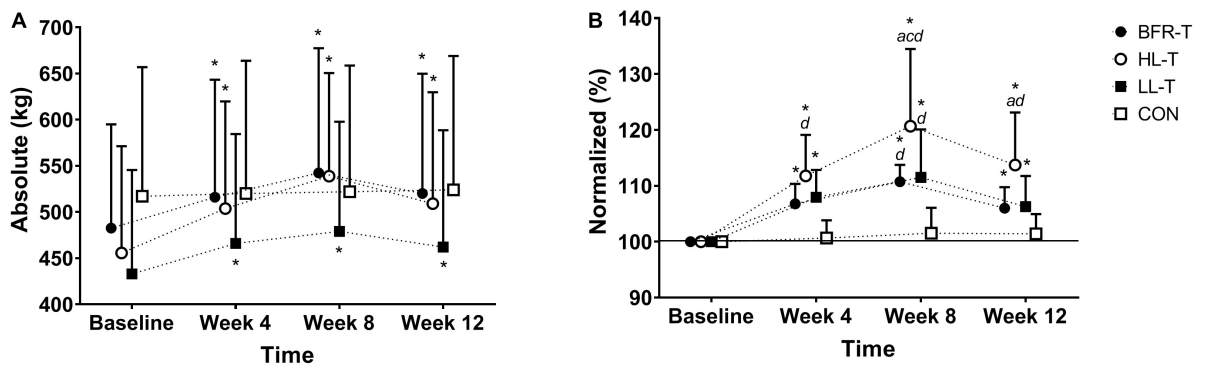

FIGURE 2 | Absolute **(A)** and Normalized **(B)** change in Total Tonnage. * indicates significant Group x Time interaction ($P \leq 0.0001$); *a* significantly different to BFR-T; *b* significant different to HL-T; *c* significantly different to LL-T; d significantly different to CON.

TABLE 3 | Body composition and muscle thickness as represented at Baseline.

	BFR-T	HL-T	LL-T	CON	Group	Time	Group x Time
Body composition (kg)							
Lean mass	52.29 ± 13.06	49.37 ± 10.47	48.54 ± 11.27	56.19 ± 10.82	0.56	**≤0.01**	0.25
Fat mass	16.71 ± 7.27	18.42 ± 6.77	17.17 ± 12.81	17.67 ± 7.81	0.96	0.42	0.48
Arm-LM	6.22 ± 1.86	6.11 ± 1.72	5.83 ± 1.94	7.17 ± 1.73	0.48	**≤0.01**	0.97
Leg-LM	17.67 ± 5.08	16.48 ± 3.59	16.31 ± 4.03	18.45 ± 3.79	0.75	0.07	0.71
Trunk-LM	24.21 ± 6.22	22.57 ± 5.29	22.13 ± 4.57	26.12 ± 4.66	0.38	0.66	0.29
Muscle thickness (cm)							
Biceps brachii	2.93 ± 0.51	2.81 ± 0.53	2.92 ± 0.62	3.22 ± 0.65	0.68	**≤0.001**	**≤0.001**
Triceps brachii	3.22 ± 0.83	3.09 ± 0.84	2.82 ± 0.83	3.42 ± 0.99	0.24	**0.02**	0.17
Pectoralis Major	1.56 ± 0.29	1.76 ± 0.30	1.61 ± 0.36	1.89 ± 0.14	0.22	0.53	0.67
Quadriceps	4.28 ± 0.52	4.39 ± 0.82	4.10 ± 0.79	4.14 ± 0.96	0.37	**≤0.001**	**0.03**
Hamstrings	5.56 ± 0.93	5.28 ± 0.85	5.37 ± 1.07	5.46 ± 0.87	0.77	**≤0.001**	0.51
Calf	5.48 ± 1.15	5.35 ± 0.97	5.48 ± 0.91	5.45 ± 0.48	0.73	**≤0.001**	0.35
Tibialis Anterior	3.01 ± 0.34	3.12 ± 0.33	3.21 ± 0.44	3.03 ± 0.37	0.72	0.52	0.50

Bold values represent significant main effects or interactions for each dependent variable.

MTH measurement sites ($P < 0.01$) except for Tibialis Anterior and Pectoralis Major. A Group x Time interaction was detected for the normalized change for Biceps brachii and Quadriceps MTH only ($P < 0.01$; **Supplementary Table 4**). At week 8, HL-T had a significantly greater percentage increase in Biceps MTH compared with baseline, which was also greater than BFR-T, while there were no other significant changes reported for the other groups. The Quadriceps MTH percent change had significantly increased at week 8 for both HL-T and LL-T only, with HL-T also being greater than CON.

Detraining

Table 2 displays the absolute (kg) strength values for the lower- and upper-body during training and detraining. Following the 4-week detraining period, absolute (kg) KE 1RM strength remained significantly elevated above baseline for BFR-T and HL-T. SQ 1RM strength was also significantly elevated above baseline for BFR-T, HL-T and LL-T. Both CR and SR strength were higher relative to baseline for HL-T only. When examining the normalized data (see **Figures 1A–F**), KE 1RM strength remained higher at week 12 for all groups except CON. In addition, the KE 1RM strength percentage change for BFR-T and HL-T was higher than CON. The SQ 1RM percentage change was higher at week 12 for all groups except CON, however, only LL-T was higher in comparison with all other groups. For the upper-body, SR 1RM strength remained higher at week 12 for HL-T in comparison with baseline, and was also higher than LL-T and CON, while BFR-T was also significantly higher than LL-T. Following the four-week detraining period, there was also a significant main effect for Time, whereby both CR and BC 1RM strength percentage change remained higher in comparison with Baseline. Overall, the absolute TT remained higher at week 12 relative to baseline for all groups except CON (**Figure 2A**). The normalized change for TT also remained higher compared with baseline at week 12 for all groups except CON, although HL-T was also higher than both BFR-T and CON (**Figure 2B**).

Supplementary Tables 1, **2** display the absolute and normalized values for all body composition data, while **Supplementary Tables 3**, **4** displays the values for MTH data. A main effect for Time was detected for BM, whereby BM was higher at week 12 relative to all other time points ($P < 0.001$). This appeared to be driven by a significant Group x Time interaction for FM whereby FM was also higher at week 12 relative to all other time points. Both HL-T and LL-T had significantly increased FM at week 12 relative to all other time points. At week 12, the percent increase in LM (0.9%) and arm-LM (1.9%) also remained higher relative to baseline levels.

For MTH (cm), a significant main effect for Time remained for Triceps, Quadriceps, Hamstring and Calf with week 12 being greater than Baseline. In addition, the Group x Time interaction remained, with Quadriceps MTH (cm) for HL-T being greater at week 12 relative to Baseline. A similar pattern was detected for MTH (%), with the percent increase for Triceps (5.3%), Quadriceps (4.9%), Hamstring (8.3%) and Calf (6.1%) being higher at week 12 relative to Baseline. In addition, the percent change for Biceps MTH was higher at week 12 for HL-T in comparison with Baseline (7.6%). Finally, the percent change for Quadriceps MTH remained higher at week 12 for HL-T in comparison with Baseline (11.5%).

DISCUSSION

The present study examined the muscular adaptations to an 8-week whole body resistance training program both with and without BFR, and the effects of a 4-week detraining period. To our knowledge, this is the first study to examine adaptations in muscle strength and mass to a whole body resistance training program with BFR and compare these to moderate-heavy load and light-load non-BFR training in a young adult population. The major findings showed that muscle strength and mass increased for BFR-T to different degrees for each exercise/muscle group, which was similar to LL-T, and overall the increase in whole body strength appeared to be higher for HL-T in comparison with all

other groups. The increase in muscle mass was similar for all training groups. Furthermore, following 4-weeks of detraining, whole body strength increases were maintained following all groups other than CON in the present study, but only the HL-T was significantly greater than the other groups. These results suggest that BFR training is an effective mode of exercise to improve muscle strength and mass when undertaken as part of a whole body program (i.e., incorporating three upper-body and three lower-body exercises), with these improvements being similar to traditional moderate-heavy load or light-load training (LL-T) without BFR.

Training Adaptations

On closer examination of the individual exercise changes in absolute 1RM strength, BFR-T produced significant increases in 5 of 6 exercises, the same as LL-T, while all 6 exercises improved for HL-T (see **Table 2**). However, examination of percent change may be more prudent to examine throughout the following discussion as it allows for a clear and relative depiction of the changes for each group.

Over the first four-week training period, the increase in KE strength as one of the main outcome measures was significantly greater for BFR-T (14%) and HL-T (15%) when compared with CON, while these were not different to LL-T despite the differing magnitude of change (8%). However, Quadriceps MTH was only increased for HL-T and not any other group. While this contrasts with previous studies that report quite rapid gains in muscle mass with BFR training [e.g., within 1–2 weeks; (Loenneke et al., 2011; Scott et al., 2014)], these studies have used greater training frequencies (1–2 times per day) and performed training to muscular failure. Continuation of the training program for a further four weeks resulted in all three groups increasing KE 1RM strength and while the percentage change for all groups was significant, only the HL-T group was greater than both LL-T and CON, while BFR-T was greater than CON but similar to LL-T.

In contrast to the present study, much of the previous literature comparing the change in absolute/normalized 1RM strength and muscle mass show greater adaptations following BFR in comparison with load matched controls (Loenneke et al., 2011). However, the typical focus of BFR literature is on "single" exercises or "dual" exercises for the lower- (Sumide et al., 2009) or upper-body (Thiebaud et al., 2013), with very few attempting under significantly variable conditions, or attempting to compare with traditional training modes (Karabulut et al., 2010; Bryk et al., 2016). While important in establishing the likely outcomes for BFR exercise training, resistance training programs should contain several exercises for both the lower- and upper-body in order maximize the gains in muscular adaptations (American College of Sports Medicine, 2009). Therefore, a major novelty of the present study was that BFR was performed during a whole body resistance training program in a young adult population. This effectively increased the total volume of work (sets x repetitions) performed across a large number of muscle groups, some of which may have been involved in more than one exercise (e.g., increased quadriceps muscle involvement with the combination of KE and SQ). Given there exists a strong possibility for a dose-response relationship between muscular adaptations and BFR resistance training volume, at least up to a certain volume (Martín-Hernández et al., 2013) it is probable that the similarities in strength and mass between the BFR-T and LL-T training groups can be explained by the increased total volume of work performed. Additionally, previous studies have shown that longer training durations ($\geq$8 weeks) produces similar improvements in muscle strength and mass for LL-T with and without BFR (Fitschen et al., 2013; Barcelos et al., 2015) which agrees with the results of the present study.

Examination of the individual exercise response to the present training program as a whole is difficult, given that each exercise increased differently between and within-groups across the 8-week training program. Therefore, we attempted to summarize the data by calculating whole body strength via TT. Overall, the percentage change in TT was highest for HL-T following the 8-week training program (21%). Further, the increase in TT was similar for BFR-T (11%) and LL-T (12%), with all groups stronger than CON (1%). In summary, whole body strength increased following training in the following manner: HL-T > BFR-T = LL-T > CON. Previous studies have observed similar adaptations in muscle strength between BFRE and moderate-HL-T (Takarada et al., 2000) while others have demonstrated lower responses for BFRE in comparison with moderate-HL-T (Lixandrão et al., 2015). The differences in results between studies could be explained by different populations, exercise selection and training protocols, and the BFR methodology being used between studies. However, a recent meta-analysis comparing the two modes of resistance training found that traditional moderate-heavy load resistance training produced a 7% advantage in strength when compared with BFR (Lixandrão et al., 2017), which is in line with the results from the present study. Interestingly, the same meta-analysis also found that muscle mass increased similarly between the two modes of exercise, which was generally observed in the results from the present study. To highlight this, there were no between-group differences across the training program for lean mass, arm- or leg-lean mass, or any of the seven MTH sites.

Based on the results of the present study as well as data from previous literature (Loenneke et al., 2011; Lixandrão et al., 2017), it appears that LL-T both with and without BFR, was less effective for the development of muscle strength in comparison with traditional moderate-HL-T and should thus question the training protocols used in order to explain our results. BFR pressures were individualized for each participant, with pressures equal to 60% LOP. While the "optimal" BFR pressure to induce maximal adaptations in muscle strength or mass is not known, 40–80% of the maximal limb/arterial occlusion pressures have been recommended previously (Patterson and Brandner, 2017), while Counts et al. (2015) recently showed similar adaptations in muscle strength and endurance following 8 weeks of BFR with either 40 or 90% of the maximal occlusion pressure. Some individuals in the present study did not increase their 1RM strength in one or more of the exercises at the end of week 4, and thus a progressive overload stimulus was not applied for the next four-week training period. For the BFR-T and LL-T groups, if the training loads (kg) lifted progressed in each training period, or each week were progressively overloaded (e.g.,

20, 30, then 40% 1RM), then perhaps greater gains in both muscle strength and mass may have been observed and this would likely reflect what would occur in a *real world* training program or rehabilitation setting. BFRE has previously been shown to be effective at increasing muscular endurance and hypertrophy with loads as low as 15% MVIC (Kacin and Strazar, 2011), but this same load is likely not sufficient for maximizing 1RM strength without training at a load closer to maximal intensity (Jessee et al., 2018). Although in some instances, such improvements have been shown to occur between loads ranging from 20 to 50% 1RM (Barcelos et al., 2015). Based on this information, while it is likely that participants were training at a sufficient BFR pressure and training load throughout the present study, there may be some degree of load-specificity required to maximize strength adaptations. Consequently, low-load whole body resistance training with BFR may not be the best way to apply this technique in younger adults if the aim of resistance training is to improve muscle strength. BFRE may be better suited for populations who are not contraindicated to lifting moderate-heavy loads (i.e., young, healthy adults, athletic populations) as a supplement to their regular training at the end of their workouts (Yamanaka et al., 2012; Luebbers et al., 2014). Another alternative would be to combine traditional moderate-HL-T with BFRE throughout a periodized training week, a method which has been shown to be more effective than BFRE alone (Yasuda et al., 2010). While the results of this study support the use of moderate-HL-T to develop muscle strength and mass in young healthy untrained populations, it may be expected that for individuals unable to lift heavy-loads (e.g., the elderly, following musculoskeletal injury, or where muscle atrophy and weakness occur due to the effects of inactivity or disease), that a multi-exercise program using LL-T with or without BFR may also be effective at increasing muscle strength during individual exercises and muscle mass at various anatomical sites.

Effects of Detraining

Results from the present study show that following the four-week detraining period, both KE and SQ strength remained higher in comparison with baseline for BFR-T, while there was either no change in strength for all other resistance exercises during the training period, or they returned to baseline levels. Previously, both Yasuda et al. (2015) and Yasuda et al. (2014b) demonstrated that lower body strength can be maintained for longer detraining periods (12–24 weeks) following BFR training, however, those studies were performed in older adults ($\geq$65 years). Therefore, to our knowledge this was the first study to observe that lower body strength can be maintained for short periods of detraining in a young healthy population following whole body BFR training. When the strength data was combined to calculate the TT, a metric of whole body strength, 1RM strength for BFR-T remained higher at week 12 when compared with baseline. However, it is important to note that while KE, SQ, and TT strength remained elevated, these were not different to the other groups. Overall, only the HL-T group maintained a training-related increase in whole body TT strength relative to baseline levels, which was also greater than all other groups. Similarly, the increase in strength for SR and BC observed in the present study following BFR-T was only maintained after detraining for BC, and this was also not different to the other groups. Previously, Yasuda et al. (2014b) had shown that following six weeks of bench press training with BFR in young males (22–27 years), not only had 1RM strength significantly increased by 4.3%, but this remained elevated by 4.9% following 3 weeks of detraining. Upon closer examination, the BFR-T group in the present study produced a non-significant increase in BP 1RM similarly to Yasuda et al. (2014b) by 6% following training, so it is unknown why these adaptations were not maintained in the present cohort except that we measured an additional one week of detraining.

Of the previous studies reporting the effects of detraining following BFR, while strength has been shown to be maintained, muscle mass appears to return to baseline levels despite improvements during the training program (Yasuda et al., 2014a,b). This effect was also apparent in the present study with no significant Group x Time interactions detected for BFR-T. However, there were significant main effects for Time whereby LM (0.9%), Arm-LM (1.9%), Triceps MTH (5.3%), Quadriceps MTH (4.9%), Hamstring MTH (8.3%) and Calf MTH (6.1%) all remained elevated above baseline levels. Importantly, these percentage increases appear not to be driven by the CON group, and collectively, despite the lower total volume load lifted by the BFR-T and LL-T groups in comparison to HL-T, all training groups were able to maintain some improvement in muscle mass throughout the detraining period. It should also be noted that the Quadriceps MTH for HL-T remained 12% higher at week 12, which was significantly higher than all other groups. Therefore, it is probable that changes in muscle architecture were also responsible for strength maintenance for HL-T during detraining, similar to previous literature (Narici et al., 1989; Hakkinen et al., 2000). Overall, given that both TT and several body composition and MTH measurements remained significantly elevated following detraining for HL-T, which were higher than all other groups, it appears that traditional moderate-heavy load (70% 1RM) training had the greatest effect on strength and mass maintenance across the four-week detraining period.

Limitations

The sample size in the present study, while satisfying the sample size calculations, is objectively a small sample size given the number of groups (4), testing time points (4), and multiple measures of muscle strength and mass. However, the use of linear mixed models for our statistical analysis accounts for smaller sample sizes well and also overcomes the presence of a small number of missing data points. In addition, whilst we attempted to recruit male and female participants, it was not the purpose of the current study to compare muscular adaptations between genders. Thus, given the disproportionate sample of males (27) to females (12) we attempted to balance genders across training groups and statistical comparisons were not made. We did not monitor physical exercise or nutrition outside of the study, although participants were aware that no additional resistance

training should take place throughout the study period, especially including the detraining period (including CON). As mentioned in the discussion, we did not control for a learning effect in our strength measurements. Given the recruitment of participants were novice lifters, it is possible that an initial familiarization (i.e., multiple weeks of training prior to testing) may have diminished any potential learning effect, however, this was not done in the current study.

CONCLUSION

The present study examined the change in muscle strength and mass in a young healthy population during an 8-week whole body resistance training program, as well as monitoring these adaptations following a 4-week detraining period. The results showed that whole body resistance training with BFR significantly improved lower-body and upper-body strength (overall; 11% increase in TT), however, this was similar to LL-T (12% increase in TT), but both groups were lower in comparison with traditional moderate-HL-T (21% increase in TT) and all groups greater than CON. Some markers of body composition (e.g., lean mass) and MTH significantly increased over the course of the 8-week training period, but these were similar across all groups. Finally, whole body strength remained significantly elevated following the four-week detraining period for BFR-T (6%), HL-T (14%), and LL-T (6%) but only the HL-T group remained higher than any of the other groups. Overall, a whole body resistance training program with BFR was shown to be an effective training mode to increase muscular strength during training and remain elevated following four weeks of detraining. However, the present study appears to show that resistance training with moderate-heavy loads (70% 1RM) results in greater adaptations in strength and muscle mass as well as higher levels of strength maintenance following detraining.

ETHICS STATEMENT

This study was approved by the Human Research Ethics Committee of Deakin University (project identification: HREC 2011-228), and all experiments were conducted according to the standards established by the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

CB, DK, and SW conceived and designed the research. CB conducted the experiments. CB, MC, and SW analyzed the data. All authors wrote, edited, and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01099/full#supplementary-material

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